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(54) Title: METHOD FOR DIAGNOSING PROSTATE CANCER

(57) Abstract: Objective methods for detecting and diagnosing prostate cancer (PRC) or prostatic intraepithelial neoplasia (PIN) are described herein. In one embodiment, the diagnostic method involves the determining a expression level of PRC-associated gene that discriminate between PRC or PIN and normal cell. The present invention further provides methods of screening for therapeutic agents useful in the treatment of either or both of PRC and PIN, methods of treating either or both of PRC and PIN and method of vaccinating a subject against either or both of PRC and PIN.

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DESCRIPTION

METHOD FOR DIAGNOSING PROSTATE CANCER

5 The present application is related to USSN 60/414.873, filed September 30, 2002, which is incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to methods of diagnosing prostate cancer.

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BACKGROUND OF THE INVENTION

Prostate cancer (PRC) is one of the most common malignancies in men and represents a significant worldwide health problem. It is the second most frequent cause of cancer death in the United States (1). Incidence of PRC is increasing steadily in developed countries according to the prevalence of Western-style diet and increasing number of 15 senior population. Increasing number of patients also die from this disease in Japan due to adoption of a Western life style (2). Currently, the diagnosis of PRC is based on an increased level of the serum prostate specific antigen (PSA). Early diagnosis provides an opportunity for curative surgery. Patients with organ confined PRC are usually treated and approximately 70% of them are curable with radical prostatectomy (3, 4). Most of patients 20 with advanced or relapsed disease are treated with androgen ablation therapy because growth of PRC is initially androgen dependent. Although most of these patients initially respond to androgen ablation therapy, the disease eventually progresses to androgen-independent PRC, at which point the tumor is no longer responsive to androgen ablation therapy.

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One of the most serious clinical problems of treatment for PRC is that this androgen-independent PRC is unresponsive to any other therapies, and understanding the mechanism of androgen-independent growth and establishing new therapies other than androgen ablation therapy against PRC are urgent issues for management of PRC.

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On the other hand, prostatic intraepithelial neoplasia (PIN) is the specific type of minimal lesion that is believed to be the precursor of PRC (McNeal & Bostwick, 1986). PIN is regarded as a continuum between low-grade and high-grade forms, and high-grade PIN is considered to be the immediate precursor of invasive carcinoma. High-grade PIN

and PRC frequently coexist and they share the similar chromosomal and genetic alterations (Qian et al., 1999). However, the mechanism of PIN development and the progression from PIN to PRC remain unclear. Therefore, genome-wide analysis of expression profiles in PINs is an essential step toward understanding the molecular carcinogenesis and 5 progression and the preventive strategies of PRC.

cDNA microarray technologies have enabled to obtain comprehensive profiles of gene expression in normal and malignant cells, and compare the gene expression in malignant and corresponding normal cells (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61: 3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); 10 Hasegawa et al., Cancer Res 62:7012-7 (2002)). This approach enables to disclose the complex nature of cancer cells, and helps to understand the mechanism of carcinogenesis. Identification of genes that are deregulated in tumors can lead to more precise and accurate diagnosis of individual cancers, and to develop novel therapeutic targets (Bienz and Clevers, Cell 103:311-20 (2000)). To disclose mechanisms underlying tumors from a 15 genome-wide point of view, and discover target molecules for diagnosis and development of novel therapeutic drugs, the present inventors have been analyzing the expression profiles of tumor cells using a cDNA microarray of 23040 genes (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61:3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)).

20 Studies designed to reveal mechanisms of carcinogenesis have already facilitated identification of molecular targets for anti-tumor agents. For example, inhibitors of farnesyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, has been effective in treating Ras-dependent tumors in animal models (He et al., Cell 99:335-45 (1999)). Clinical trials on human using a combination of anti-cancer drugs and anti- 25 HER2 monoclonal antibody, trastuzumab, have been conducted to antagonize the proto-oncogene receptor HER2/neu; and have been achieving improved clinical response and overall survival of breast-cancer patients (Lin et al., Cancer Res 61:6345-9 (2001)). A tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, 30 has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes. Agents of these kinds are designed to suppress oncogenic activity of specific gene products (Fujita

et al., *Cancer Res* 61:7722-6 (2001)). Therefore, gene products commonly up-regulated in cancerous cells may serve as potential targets for developing novel anti-cancer agents.

It has been demonstrated that CD8+ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC Class I molecule, and lyse tumor cells. Since the discovery of MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon, *Int J Cancer* 54: 177-80 (1993); Boon and van der Bruggen, *J Exp Med* 183: 725-9 (1996); van der Bruggen et al., *Science* 254: 1643-7 (1991); Brichard et al., *J Exp Med* 178: 489-95 (1993); Kawakami et al., *J Exp Med* 180: 347-52 (1994)). Some of the discovered TAAs are now in the stage of clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen et al., *Science* 254: 1643-7 (1991)), gp100 (Kawakami et al., *J Exp Med* 180: 347-52 (1994)), SART (Shichijo et al., *J Exp Med* 187: 277-88 (1998)), and NY-ESO-1 (Chen et al., *Proc Natl Acad Sci USA* 94: 1914-8 (1997)). On the other hand, gene products which had been demonstrated to be specifically overexpressed in tumor cells, have been shown to be recognized as targets inducing cellular immune responses. Such gene products include p53 (Umano et al., *Brit J Cancer* 84: 1052-7 (2001)), HER2/neu (Tanaka et al., *Brit J Cancer* 84: 94-9 (2001)), CEA (Nukaya et al., *Int J Cancer* 80: 92-7 (1999)), and so on.

In spite of significant progress in basic and clinical research concerning TAAs (Rosenbeg et al., *Nature Med* 4: 321-7 (1998); Mukherji et al., *Proc Natl Acad Sci USA* 92: 8078-82 (1995); Hu et al., *Cancer Res* 56: 2479-83 (1996)), only limited number of candidate TAAs for the treatment of adenocarcinomas, including colorectal cancer, are available. TAAs abundantly expressed in cancer cells, and at the same time which expression is restricted to cancer cells would be promising candidates as immunotherapeutic targets. Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategy in various types of cancer (Boon and can der Bruggen, *J Exp Med* 183: 725-9 (1996); van der Bruggen et al., *Science* 254: 1643-7 (1991); Brichard et al., *J Exp Med* 178: 489-95 (1993); Kawakami et al., *J Exp Med* 180: 347-52 (1994); Shichijo et al., *J Exp Med* 187: 277-88 (1998); Chen et al., *Proc Natl Acad Sci USA* 94: 1914-8 (1997); Harris, *J Natl Cancer Inst* 88: 1442-5 (1996); Butterfield et al., *Cancer Res* 59: 3134-42 (1999); Vissers et al., *Cancer Res* 59: 5554-9 (1999); van der Burg et al., *J*

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Immunol 156: 3308-14 (1996); Tanaka et al., Cancer Res 57: 4465-8 (1997); Fujie et al., Int J Cancer 80: 169-72 (1999); Kikuchi et al., Int J Cancer 81: 459-66 (1999); Oiso et al., Int J Cancer 81: 387-94 (1999)).

It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN- γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or -A0201 restricted manner in ^{51}Cr -release assays (Kawano et al., Cance Res 60: 3550-8 (2000); Nishizaka et al., Cancer Res 60: 4830-7 (2000); Tamura et al., Jpn J Cancer Res 92: 762-7 (2001)). However, both of HLA-A24 and HLA-A0201 are one of the popular HLA alleles in Japanese, as well as Caucasian (Date et al., Tissue Antigens 47: 93-101 (1996); Kondo et al., J Immunol 155: 4307-12 (1995); Kubo et al., J Immunol 152: 3913-24 (1994); Imanishi et al., Proceeding of the eleventh International Hictocompatibility Workshop and Conference Oxford University Press, Oxford, 1065 (1992); Williams et al., Tissue Antigen 49: 129 (1997)). Thus, antigenic peptides of carcinomas presented by these HLAs may be especially useful for the treatment of carcinomas among Japanese and Caucasian. Further, it is known that the induction of low-affinity CTL in vitro usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., Proc Natl Acad Sci USA 93: 4102-7 (1996)).

SUMMARY OF THE INVENTION

The invention is based on the discovery of a pattern of gene expression correlated with PRC or PIN. The genes that are differentially expressed in either or both of PRC and PIN are collectively referred to herein as "PRC nucleic acids" or "PRC polynucleotides" and the corresponding encoded polypeptides are referred to as "PRC polypeptides" or "PRC proteins."

Accordingly, the invention features a method of diagnosing or determining a predisposition to either or both of PRC and PIN in a subject by determining an expression level of a PRC-associated gene in a patient derived biological sample, such as tissue sample. By PRC associated gene is meant a gene that is characterized by an expression

level which differs in a cell obtained from a PRC or PIN cell compared to a normal cell. A normal cell is one obtained from prostate tissue. A PRC-associated gene includes for example PRC 1-692. An alteration, *e.g.*, increase or decrease of the level of expression of the gene compared to a normal control level of the gene indicates that the subject suffers 5 from or is at risk of developing either or both of PRC and PIN.

By normal control level is meant a level of gene expression detected in a normal, healthy individual or in a population of individuals known not to be suffering from PRC and PIN. A control level is a single expression pattern derived from a single reference population or from a plurality of expression patterns. For example, the control level can be 10 a database of expression patterns from previously tested cells. A normal individual is one with no clinical symptoms of PRC and PIN.

An increase in the level of PRC 1-88,296-321,458-537 detected in a test sample compared to a normal control level indicates the subject (from which the sample was obtained) suffers from or is at risk of developing at least either of PRC or PIN. In contrast, 15 a decrease in the level of PRC 89-295,322-457,538-692 detected in a test sample compared to a normal control level indicates said subject suffers from or is at risk of developing either or both of PRC and PIN.

Alternatively, expression of a panel of PRC-associated genes in the sample is compared to a PRC control level of the same panel of genes. By PRC control level is 20 meant the expression profile of the PRC-associated genes found in a population suffering from either or both of PRC and PIN.

Gene expression is increased or decreased 10%, 25%, 50% compared to the control level. Alternately, gene expression is increased or decreased 1, 2, 5 or more fold compared to the control level. Expression is determined by detecting hybridization, *e.g.*, on an array, 25 of a PRC-associated gene probe to a gene transcript of the patient-derived tissue sample.

The patient derived tissue sample is any tissue from a test subject, *e.g.*, a patient known to or suspected of having PRC or PIN. For example, the tissue contains an epithelial cell. For example, the tissue is an epithelial cell from prostate tissue.

The invention also provides a PRC reference expression profile of a gene expression level of two or more of PRC 1-692. Alternatively, the invention provides a 30 PRC reference expression profile of the levels of expression two or more of PRC 1-88,

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PRC 89-295, PRC 296-321, PRC 322-457, PRC 458-537, or PRC 538-692.

The invention further provides methods of identifying an agent that inhibits or enhances the expression or activity of a PRC-associated gene, e.g. PRC 1-692 by contacting a test cell expressing a PRC associated gene with a test agent and determining the expression level of the PRC associated gene. The test cell is an epithelial cell such as an epithelial cell from prostate tissue. A decrease of the level compared to a normal control level of the gene indicates that the test agent is an inhibitor of the PRC-associated gene and reduces a symptom of either or both of PRC and PIN. Alternatively, an increase of the level or activity compared to a normal control level or activity of the gene indicates that said test agent is an enhancer of expression or function of the PRC associated gene and reduces a symptom of either or both of PRC and PIN, e.g., PRC 89-295, PRC 322-457, PRC 538-692.

The invention also provides a kit with a detection reagent which binds to two or more PRC nucleic acid sequences or which binds to a gene product encoded by the nucleic acid sequences. Also provided is an array of nucleic acids that binds to two or more PRC nucleic acids.

Therapeutic methods include a method of treating or preventing either or both of PRC and PIN in a subject by administering to the subject an antisense composition. The antisense composition reduces the expression of a specific target gene, e.g., the antisense composition contains a nucleotide, which is complementary to a sequence selected from the group consisting of PRC 1-88, 296-321, 458-537. Another method includes the steps of administering to a subject an small interfering RNA (siRNA) composition. The siRNA composition reduces the expression of a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537. In yet another method, treatment or prevention of either or both of PRC and PIN in a subject is carried out by administering to a subject a ribozyme composition. The nucleic acid-specific ribozyme composition reduces the expression of a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537. Other therapeutic methods include those in which a subject is administered a compound that increases the expression of PRC 89-295, 322-457, 538-692 or activity of a polypeptide encoded by PRC 89-295, 322-457, 538-692. Furthermore, either or both of PRC and PIN can be treated by administering a protein encoded by PRC 89-295, 322-457, 538-692. The protein may be directly administered to the patient or, alternatively, may be expressed in

vivo subsequent to being introduced into the patient, for example, by administering an expression vector or host cell carrying the down-regulated marker gene of interest. Suitable mechanisms for in vivo expression of a gene of interest are known in the art.

The invention also includes vaccines and vaccination methods. For example, a 5 method of treating or preventing either or both of PRC and PIN in a subject is carried out by administering to the subject a vaccine containing a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537 or an immunologically active fragment such a polypeptide. An immunologically active fragment is a polypeptide that is shorter in length than the full-length naturally-occurring 10 protein and which induces an immune response. For example, an immunologically active fragment at least 8 residues in length and stimulates an immune cell such as a T cell or a B cell. Immune cell stimulation is measured by detecting cell proliferation, elaboration of cytokines (e.g., IL-2), or production of an antibody.

Unless otherwise defined, all technical and scientific terms used herein have the 15 same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In 20 case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

One advantage of the methods described herein is that the disease is identified prior to detection of overt clinical symptoms. Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

25

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a photograph of a DNA agarose gel showing expression of representative 5 genes and β -actin examined by semi-quantitative RT-PCR using cDNA prepared from amplified RNA. Gene symbols are noted. T and N indicate tumors and normal, respectively for each of 8 patients.

DETAILED DESCRIPTION

The present invention is based in part on the discovery of changes in expression patterns of multiple nucleic acid sequences in epithelial cells of patients with PRC or PIN. The differences in gene expression were identified by using a comprehensive cDNA 5 microarray system.

cDNA microarray is a powerful tool for identifying genes that may be applicable for development of novel molecular targets for therapeutic purposes (Ishiguro et al., 2002; Yagyu et al., 2002). Now basic research about PRC are rapidly progressed recently by using genome information and new technologies, but most difficulty in studying human 10 PRCs with histological heterogeneity is the inability to isolate pure samples for molecular analysis. Most of the previous studies have used bulk cancer tissues without eliminating contamination by non-cancerous cells including stroma cells, microvasculature cells, fibromuscular cells, inflammatory cells and other epithelial cells from benign lesions including PINs. However, laser microdissection allows us to overcome this hurdle and 15 enables the precise evaluation of pure cell populations (Emmert-Buck et al., 1996) for PRC and PIN cells. Also, we compared gene expression of cancer cells with their corresponding normal epithelial cells as a control in each case. This procedure prevents individuality of gene expression from effecting on data. This study is the first report about precise expression profiles of PRCs and PINs, coupling with LMM. These data 20 would provide important information on prostatic carcinogenesis and would be greatly useful to identify candidate genes whose products can be targeted for drug design for treatment and prevention of PRC.

The gene-expression profiles of cancer cells from 20 PRCs and 10 PINs were analyzed using cDNA microarray representing 23,040 genes coupled with laser 25 microdissection. By comparing expression patterns between cancer cells from diagnostic PRC patients and normal epithelial cells purely selected with Laser Microdissection, 88 genes were identified as commonly up-regulated in PRC and PIN cells, and 207 genes were identified as being commonly down-regulated in PRC and PIN cells. 26 genes were identified as commonly up-regulated in PRC cells, and 136 genes were identified as being 30 commonly down-regulated in PRC cells. 80 genes were identified as commonly up-regulated in PIN cells and 155 genes were identified as being commonly down-regulated in

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PIN cells. In addition, selection was made of candidate molecular markers with the potential of detecting cancer-related proteins in serum or sputum of patients, and discovered some potential targets for development of signal-suppressing strategies in human PRC or PIN.

5 The differentially expressed genes identified herein are used for diagnostic purposes as markers of PRC or PIN and as gene targets, the expression of which is altered to treat or alleviate a symptom of PRC or PIN.

10 The genes whose expression levels are modulated (*i.e.*, increased or decreased) in either or both of PRC and PIN patients are summarized in Tables 3-8 and are collectively referred to herein as "PRC-associated genes", "PRC nucleic acids" or "PRC polynucleotides" and the corresponding encoded polypeptides are referred to as "PRC polypeptides" or "PRC proteins." Unless indicated otherwise, "PRC" is meant to refer to any of the sequences disclosed herein. (*e.g.*, PRC 1-692). The genes that have been previously described are presented along with a database accession number.

15 By measuring expression of the various genes in a sample of cells, PRC and PIN are diagnosed. Similarly, by measuring the expression of these genes in response to various agents, agents for treating either or both of PRC and PIN can be identified.

20 The invention involves determining (*e.g.*, measuring) the expression of at least one, and up to all the PRC sequences listed in Tables 3-8. Using sequence information provided by the GeneBankTM database entries for the known sequences the PRC associated genes are detected and measured using techniques well known to one of ordinary skill in the art. For example, sequences within the sequence database entries corresponding to PRC sequences, are used to construct probes for detecting PRC RNA sequences in, *e.g.*, northern blot hybridization analyses. Probes include at least 10, 20, 50, 100, 200
25 nucleotides of a reference sequence. As another example, the sequences can be used to construct primers for specifically amplifying the PRC nucleic acid in, *e.g.*, amplification-based detection methods such as reverse-transcription based polymerase chain reaction.

30 Expression level of one or more of the PRC-associated genes in the test cell population, *e.g.*, a patient derived tissues sample is then compared to expression levels of the some genes in a reference population. The reference cell population includes one or more cells for which the compared parameter is known, *i.e.*, PRC cells or non-PRC cells.

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Whether or not a pattern of gene expression in the test cell population compared to the reference cell population indicates PRC or PIN, or a predisposition thereto depends upon the composition of the reference cell population. For example, if the reference cell population is composed of non- PRC cells, a similar gene expression pattern in the test cell 5 population and reference cell population indicates the test cell population is non-PRC. Conversely, if the reference cell population is made up of PRC cells, a similar gene expression profile between the test cell population and the reference cell population that the test cell population includes PRC cells.

A level of expression of a PRC marker gene in a test cell population is considered 10 altered in levels of expression if its expression level varies from the reference cell population by more than 1.0, 1.5, 2.0, 5.0, 10.0 or more fold from the expression level of the corresponding PRC marker gene in the reference cell population.

Differential gene expression between a test cell population and a reference cell population is normalized to a control nucleic acid, *e.g.* a housekeeping gene. For example, 15 a control nucleic acid is one which is known not to differ depending on the PRC or non-PRC state of the cell. Expression levels of the control nucleic acid in the test and reference nucleic acid can be used to normalize signal levels in the compared populations. Control genes include β -actin, glyceraldehyde 3- phosphate dehydrogenase or ribosomal protein P1.

The test cell population is compared to multiple reference cell populations. Each of 20 the multiple reference populations may differ in the known parameter. Thus, a test cell population may be compared to a second reference cell population known to contain, *e.g.*, PRC cells, as well as a second reference population known to contain, *e.g.*, non-PRC cells (normal cells). The test cell is included in a tissue type or cell sample from a subject known to contain, or to be suspected of containing, PRC cells.

25 The test cell is obtained from a bodily tissue or a bodily fluid, *e.g.*, biological fluid (such as blood, or serum). For example, the test cell is purified from a tissue. Preferably, the test cell population comprises a epithelial cell. The epithelial cell is from tissue known to be or suspected to be cancerous.

Cells in the reference cell population are derived from a tissue type as similar to 30 test cell. Optionally, the refernce cell poulation is a cell line, *e.g.* a PRC cell line (positive control) or a norma non-PRC cell line (negative control). Alternatively, the control cell

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population is derived from a database of molecular information derived from cells for which the assayed parameter or condition is known.

The subject is preferably a mammal. The mammal can be, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

5 Expression of the genes disclosed herein is determined at the protein or nucleic acid level using methods known in the art. For example, Northern hybridization analysis using probes which specifically recognize one or more of these nucleic acid sequences can be used to determine gene expression. Alternatively, expression is measured using reverse-transcription-based PCR assays, *e.g.*, using primers specific for the differentially expressed
10 gene sequences. Expression is also determined at the protein level, *i.e.*, by measuring the levels of polypeptides encoded by the gene products described herein, or biological activity thereof. Such methods are well known in the art and include, *e.g.*, immunoassays based on antibodies to proteins encoded by the genes. The biological activity of the proteins encoded by the genes are also well known.

15 *Diagnosing PRC or PIN*

PRC or PIN is diagnosed by measuring the expression level of one or more PRC nucleic acid sequences from a test population of cells, (*i.e.*, a patient derived biological sample). Preferably, the test cell population comprises an epithelial cell, *e.g.*, a cell obtained from prostate tissue. Gene expression is also measured from blood or other
20 bodily fluids such as urine. Other biological samples can be used for measuring the protein level. For example, the protein level in the blood, or serum derived from subject to be diagnosed can be measured by immunoassay or biological assay.

25 Expression of one or more of an PRC-associated gene, *e.g.*, PRC 1-692 is determined in the test cell or biological sample and compared to the expression of the normal control level. A normal control level is an expression profile of a PRC-associated gene typically found in a population known not to be suffering from PRC. An increase or a decrease of the level of expression in the patient derived tissue sample of the PRC associated genes indicates that the subject is suffering from or is at risk of developing PRC or PIN. For example, an increase in expression of PRC 1-88, PRC 296-321, PRC 458-537
30 in the test population compared to the normal control level indicates that the subject is

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suffering from or is at risk of developing PRC or PIN. Conversely, a decrease in expression of PRC 89-295, PRC 322-457, PRC 538-692 in the test population compared to the normal control level indicates that the subject is suffering from or is at risk of developing PRC or PIN.

5 When one or more of the PRC-associated genes are altered in the test population compared to the normal control level indicates that the subject suffers from or is at risk of developing PRC or PIN. For example, at least 1%, 5%, 25%, 50%, 60%, 80%, 90% or more of the panel of PRC-associated genes (PRC 1-88, PRC 296-321, PRC 458-537, PRC 89-295, PRC 322-457, or PRC 538-692) are altered.

10 The expression levels of the PRC 1-692 in a particular specimen can be estimated by quantifying mRNA corresponding to or protein encoded by PRC 1-692. Quantification methods for mRNA are known to those skilled in the art. For example, the levels of mRNAs corresponding to the PRC 1-692 can be estimated by Northern blotting or RT-PCR. Since the nucleotide sequence of the PRC 1-692 have already been reported.

15 Anyone skilled in the art can design the nucleotide sequences for probes or primers to quantify the PRC 1-692.

Also the expression level of the PRC 1-692 can be analyzed based on the activity or quantity of protein encoded by the gene. A method for determining the quantity of the PRC 1-692 protein is shown in bellow. For example, immunoassay method is useful for 20 the determination of the proteins in biological materials. Any biological materials can be used for the determination of the protein or it's activity. For example, blood sample is analyzed for estimation of the protein encoded by a serum marker. On the other hand, a suitable method can be selected for the determination of the activity of a protein encoded by the PRC 1-692 according to the activity of each protein to be analyzed.

25 In the present invention, a diagnostic agent for diagnosing PRC or PIN, is also provided. The diagnostic agent of the present invention comprises a compound that binds to a polynucleotide or a polypeptide of the present invention. Preferably, an oligonucleotide that hybridizes to the polynucleotide of the PRC 1-692, or an antibody that binds to the polypeptide of the PRC 1-692 may be used as such a compound.

30 In the present invention, PRC 1-692 are useful for diagnosing either or both of PRC and PIN. PRC 1-295 are useful for diagnosing both of PRC and PIN. PRC 296-457 are also useful for diagnosing PRC as PRC specific markers. Furthermore, PRC 458-692 are

useful for diagnosing PIN as PIN specific markers.

Identifying Agents that inhibit or enhance PRC-associated gene expression

An agent that inhibits the expression or activity of an PRC-associated gene is

5 identified by contacting a test cell population expressing an PRC associated upregulated gene with a test agent and determining the expression level of the PRC associated gene. A decrease in expression in the presence of the agent compared to the normal control level (or compared to the level in the absence of the test agent) indicates the agent is an inhibitor of an PRC associated upregulated gene and useful to inhibit PRC or PIN.

10 Alternatively, an agent that enhances the expression or activity of an PRC downregulated associated gene is identified by contacting a test cell population expressing an PRC associated gene with a test agent and determining the expression level or activity of the PRC associated downregulated gene. An increase of expression or activity compared to a normal control expression level or activity of the PRC-associated gene

15 indicates that the test agent augments expression or activity of the downregulated PRC associated gene.

The test cell population is any cell expressing the PRC-associated genes. For example, the test cell population contains an epithelial cell, such as a cell is or derived from prostate. For example, the test cell is immortalized cell line derived from a PRC cell.

20 Alternatively, the test cell is a cell, which has been transfected with a PRC-associated gene or which has been transfected with a regulatory sequence (e.g. promoter sequence) from a PRC-associated gene operably linked to a reporter gene.

Assessing efficacy of treatment of PRC or PIN in a subject

The differentially expressed PRC-associated gene identified herein also allow for

25 the course of treatment of either or both of PRC and PIN to be monitored. In this method, a test cell population is provided from a subject undergoing treatment for PRC or PIN. If desired, test cell populations are obtained from the subject at various time points before, during, or after treatment. Expression of one or more of the PRC-associated gene, in the cell population is then determined and compared to a reference cell population which

30 includes cells whose PRC state is known. The reference cells have not been exposed to the treatment.

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If the reference cell population contains no PRC cells, a similarity in expression between PRC-associated gene in the test cell population and the reference cell population indicates that the treatment is efficacious. However, a difference in expression between PRC -associated gene in the test population and a normal control reference cell population 5 indicates the a less favorable clinical outcome or prognosis.

By "efficacious" is meant that the treatment leads to a reduction in expression of a pathologically upregulated gene, increase in expression of a pathologically down-regulated gene or a decrease in size, prevalence, or metastatic potential of PRC in a subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards 10 or prevents a PRC or PIN from forming or retards, prevents, or alleviates a symptom of clinical PRC or PIN. Assesment of prostate tumors are made using standard clinical protocols.

Efficaciousness is determined in association with any known method for diagnosing or treating either or both of PRC and PIN. PRC is diagnosed for example, by 15 identifying symptomatic anomalies, e.g., urinary symptoms such as difficulty in starting or stopping the stream, dysuria, frequency, or hematuria.

Selecting a therapeutic agent for treating PRC or PIN that is appropriate for a particular individual

Differences in the genetic makeup of individuals can result in differences in their 20 relative abilities to metabolize various drugs. An agent that is metabolized in a subject to act as an inhibitor of PRC or PIN can manifest itself by inducing a change in gene expression pattern in the subject's cells from that characteristic of an PRC state to a gene expression pattern characteristic of a non-PRC state. Accordingly, the differentially expressed PRC-associated gene disclosed herein allow for a putative therapeutic or 25 prophylactic inhibitor of PRC or PIN to be tested in a test cell population from a selected subject in order to determine if the agent is a suitable PRC or PIN inhibitor in the subject.

To identify a inhibitor of PRC or PIN, that is appropriate for a specific subject, a test cell population from the subject is exposed to a therapeutic agent, and the expression of one or more of PRC 1-692 genes is determined.

30 The test cell population contains a PRC or PIN cell expressing a PRC associated gene. Preferably, the test cell is an epithelial cell. For example a test cell population is incubated in the presence of a candidate agent and the pattern of gene expression of the test

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sample is measured and compared to one or more reference profiles, e.g., an PRC reference expression profile or an non-PRC reference expression profile.

A decrease in expression of one or more of PRC 1-88, PRC 296-321, PRC 458-537 or an increase in expression of one or more of PRC 89-295, PRC 322-457, PRC 538-692 in 5 a test cell population relative to a reference cell population containing PRC is indicative that the agent is therapeutic.

The test agent can be any compound or composition. For example, the test agents are immunomodulatory agents.

Screening assays for identifying therapeutic agents

10 The differentially expressed genes disclosed herein can also be used to identify candidate therapeutic agents for treating PRC or PIN. The method is based on screening a candidate therapeutic agent to determine if it converts an expression profile of PRC 1-692 characteristic of an PRC state to a pattern indicative of a non-PRC state.

15 In the present invention, PRC 1-692 are useful for screening of therapeutic agent for treating or preventing either or both of PRC and PIN. PRC 1-295 are used for screening of therapeutic agent for treating or preventing both of PRC and PIN. PRC 296-457 are also used as PRC specific markers for screening of therapeutic agent for treating or preventing PRC. Furthermore, PRC 458-692 are used as PIN specific markers for screening of therapeutic agent for treating or preventing PIN or preventing PRC.

20 In the method, a cell is exposed to a test agent or a combination of test agents (sequentially or consequentially) and the expression of one or more PRC 1-692 in the cell is measured. The expression profile of the PRC-associated gene in the test population is compared to expression level of the PRC-associated gene in a reference cell population that is not exposed to the test agent.

25 An agent effective in stimulating expression of underexpressed genes, or in suppressing expression of overexpressed genes is deemed to lead to a clinical benefit such compounds are further tested for the ability to prevent PRC in animals or test subjects.

30 In a further embodiment, the present invention provides methods for screening candidate agents which are potential targets in the treatment or prevention of either or both of PRC and PIN. As discussed in detail above, by controlling the expression levels or activities of marker genes, one can control the onset and progression of either or both of PRC and PIN. Thus, candidate agents, which are potential targets in the treatment or

prevention of either or both of PRC and PIN, can be identified through screenings that use the expression levels and activities of marker genes as indices. In the context of the present invention, such screening may comprise, for example, the following steps:

- a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-692; ;
- b) detecting the binding activity between the polypeptide and the test compound; and
- c) selecting a compound that binds to the polypeptide

Alternatively, the screening method of the present invention may comprise the following steps:

- a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 1-692; and
- b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 1-88, 296-321, 458-537, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 89-295,322-457,538-692.

Cells expressing a marker gene include, for example, cell lines established from PRC; such cells can be used for the above screening of the present invention.

Alternatively, the screening method of the present invention may comprise the following steps:

- a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of selected from the group consisting of PRC 1-692;
- b) detecting the biological activity of the polypeptide of step (a); and
- c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537 in comparison with the biological activity detected in the absence of the test compound, or enhances the the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 89-295,322-457,538-692 in comparison with the biological activity detected in the absence of the test compound.

A protein required for the screening can be obtained as a recombinant protein using

the nucleotide sequence of the marker gene. Based on the information of the marker gene, one skilled in the art can select any biological activity of the protein as an index for screening and a measurement method based on the selected biological activity.

5 Alternatively, the screening method of the present invention may comprise the following steps:

- a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from 10 the group consisting of PRC 1-692
- b) measuring the activity of said reporter gene; and
- c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group 15 consisting of PRC 1-88, 296-321, 458-537 as compared to a control, or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 89-295,322-457,538-692, as compared to a control.

Suitable reporter genes and host cells are well known in the art. The reporter construct required for the screening can be prepared by using the transcriptional regulatory region of 20 a marker gene. When the transcriptional regulatory region of a marker gene has been known to those skilled in the art, a reporter construct can be prepared by using the previous sequence information. When the transcriptional regulatory region of a marker gene remains unidentified, a nucleotide segment containing the transcriptional regulatory region 25 can be isolated from a genome library based on the nucleotide sequence information of the marker gene.

The compound isolated by the screening is a candidate for drugs that inhibit the activity of the protein encoded by marker genes and can be applied to the treatment or prevention of PRC or PIN.

Moreover, compound in which a part of the structure of the compound inhibiting 30 the activity of proteins encoded by marker genes is converted by addition, deletion and/or replacement are also included in the compounds obtainable by the screening method of the present invention.

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When administrating the compound isolated by the method of the invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pigs, rabbits, cats, dogs, sheep, pigs, cattle, monkeys, baboons, and chimpanzees, the isolated compound can be directly administered or can be formulated into a dosage form using known pharmaceutical preparation methods. For example, according to the need, the drugs can be taken orally, as sugar-coated tablets, capsules, elixirs and microcapsules, or non-orally, in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds can be mixed with pharmaceutically acceptable carriers or media, specifically, sterilized water, physiological saline, plant-oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binders, and such, in a unit dose form required for generally accepted drug implementation. The amount of active ingredients in these preparations makes a suitable dosage within the indicated range acquirable.

Examples of additives that can be mixed to tablets and capsules are, binders such as gelatin, corn starch, tragacanth gum and arabic gum; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose or saccharin; and flavoring agents such as peppermint, Gaultheria adenothrix oil and cherry. When the unit-dose form is a capsule, a liquid carrier, such as an oil, can also be further included in the above ingredients. Sterile composites for injections can be formulated following normal drug implementations using vehicles such as distilled water used for injections.

Physiological saline, glucose, and other isotonic liquids including adjuvants, such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride, can be used as aqueous solutions for injections. These can be used in conjunction with suitable solubilizers, such as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

Sesame oil or Soy-bean oil can be used as a oleaginous liquid and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizer and may be formulated with a buffer, such as phosphate buffer and sodium acetate buffer; a pain-killer, such as procaine hydrochloride; a stabilizer, such as benzyl alcohol and phenol; and an anti-oxidant. The prepared injection may be filled into a suitable ampule.

Methods well known to one skilled in the art may be used to administer the

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pharmaceutical composition of the present invention to patients, for example as intraarterial, intravenous, or percutaneous injections and also as intranasal, transbronchial, intramuscular or oral administrations. The dosage and method of administration vary according to the body-weight and age of a patient and the administration method; however, 5 one skilled in the art can routinely select a suitable method of administration. If said compound is encodable by a DNA, the DNA can be inserted into a vector for gene therapy and the vector administered to a patient to perform the therapy. The dosage and method of administration vary according to the body-weight, age, and symptoms of the patient but one skilled in the art can suitably select them.

10 For example, although the dose of a compound that binds to the protein of the present invention and regulates its activity depends on the symptoms, the dose is about 0.1 mg to about 100 mg per day, preferably about 1.0 mg to about 50 mg per day and more preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult (weight 60 kg).

15 When administering parenterally, in the form of an injection to a normal adult (weight 60 kg), although there are some differences according to the patient, target organ, symptoms and method of administration, it is convenient to intravenously inject a dose of about 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day. Also, in the case of other animals too, it 20 is possible to administer an amount converted to 60 kgs of body-weight.

Assessing the prognosis of a subject with PRC or PIN

Also provided is a method of assessing the prognosis of a subject with PRC or PIN by comparing the expression of one or more PRC-associated genes in a test cell population to the expression of the genes in a reference cell population derived from 25 patients over a spectrum of disease stages. By comparing gene expression of one or more PRC-associated gene in the test cell population and the reference cell population(s), or by comparing the pattern of gene expression over time in test cell populations derived from the subject, the prognosis of the subject can be assessed.

A decrease in expression of one or more of PRC 89-295, PRC 322-457, PRC 538-30 692 compared to a normal control or an increase of expression of one or more of PRC 1-88, PRC 296-321, PRC 458-537 compared to a normal control indicates less favorable prognosis. An increase in expression of one or more of PRC 89-295, PRC 322-457, PRC

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538-692 indicates a more favorable prognosis, and a decrease in expression of PRC 1-88, PRC 296-321, PRC 458-537 indicates a more favorable prognosis for the subject.

Kits

The invention also includes an PRC-detection reagent, e.g., a nucleic acid that 5 specifically binds to or identifies one or more PRC nucleic acids such as oligonucleotide sequences, which are complementary to a portion of an PRC nucleic acid or antibodies which bind to proteins encoded by an PRC nucleic acid. The reagents are packaged together in the form of a kit. The reagents are packaged in separate containers, e.g., a nucleic acid or antibody (either bound to a solid matrix or packaged separately with 10 reagents for binding them to the matrix), a control reagent (positive and/or negative), and/or a detectable label. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay are included in the kit. The assay format of the kit is a Northern hybridization or a sandwich ELISA known in the art.

For example, PRC detection reagent, is immobilized on a solid matrix such as a 15 porous strip to form at least one PRC detection site. The measurement or detection region of the porous strip may include a plurality of sites containing a nucleic acid. A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites are located on a separate strip from the test strip. Optionally, the different detection sites may contain different amounts of immobilized nucleic acids, *i.e.*, a higher amount in the first 20 detection site and lesser amounts in subsequent sites. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of PRC present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a teststrip.

25 Alternatively, the kit contains a nucleic acid substrate array comprising one or more nucleic acid sequences. The nucleic acids on the array specifically identify one or more nucleic acids represented by PRC 1-692. The expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-692 are identified by virtue if the level of binding to an array test strip or chip. The substrate array can be on, *e.g.*, a 30 solid substrate, *e.g.*, a "chip" as described in U.S. Patent No.5,744,305.

Arrays and pluralities

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The invention also includes a nucleic acid substrate array comprising one or more nucleic acid. The nucleic acids on the array specifically corresponds to one or more nucleic acid sequences represented by PRC 1-692. The level expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-692 are 5 identified by detecting nucleic acid binding to the array.

The invention also includes an isolated plurality (*i.e.*, a mixture if two or more nucleic acids) of nucleic acids. The nucleic acids are in a liquid phase or a solid phase, *e.g.*, immobilized on a solid support such as a nitrocellulose membrane. The plurality includes one or more of the nucleic acids represented by PRC 1-692. In various embodiments, the 10 plurality includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-692.

Methods of inhibiting PRC or PIN

The invention provides a method for treating or alleviating a symptom of PRC or PIN in a subject by decreasing expression or activity of PRC 1-88, PRC 296-321, PRC 15 458-537 or increasing expression or activity of PRC 89-295, PRC 322-457, PRC 538-692. Therapeutic compounds are administered prophylactically or therapeutically to subject suffering from at risk of (or susceptible to) developing PRC or PIN. Such subjects are identified using standard clinical methods or by detecting an aberrant level of expression or activity of (*e.g.*, PRC 1-692). Therapeutic agents include inhibitors of cell cycle 20 regulation, cell proliferation, and protein kinase activity.

In the present invention, PRC 1-692 are useful for treating or preventing either or both of PRC and PIN as molecular target. PRC 1-295 are useful for treating or preventing both of PRC and PIN. PRC 296-457 are also useful for treating or preventing PRC as molecular target. Furthermore, PRC 458-692 are useful for treating or preventing PIN and 25 ultimately preventing PRC.

The therapeutic method includes increasing the expression, or function, or both of one or more gene products of genes whose expression is decreased ("underexpressed genes") in PRC or PIN cell relative to normal cells of the same tissue type from which the PRC or PIN cells are derived. In these methods, the subject is treated with an effective 30 amount of a compound, which increases the amount of one of more of the underexpressed genes in the subject. Administration can be systemic or local. Therapeutic compounds include a polypeptide product of an underexpressed gene, or a biologically active fragment

thereof a nucleic acid encoding an underexpressed gene and having expression control elements permitting expression in the PRC or PIN cells; for example an agent which increases the level of expression of such gene endogenous to the PRC or PIN cells (i.e., which up-regulates expression of the underexpressed gene or genes). Administration of 5 such compounds counter the effects of aberrantly-under expressed of the gene or genes in the subject's prostate cells and improves the clinical condition of the subject.

The method also includes decreasing the expression, or function, or both, of one or more gene products of genes whose expression is aberrantly increased ("overexpressed gene") in. Expression is inhibited in any of several ways known in the art. For example, 10 expression is inhibited by administering to the subject a nucleic acid that inhibits, or antagonizes, the expression of the overexpressed gene or genes, e.g., an antisense oligonucleotide or small interfering RNA which disrupts expression of the overexpressed gene or genes.

Alternatively, function of one or more gene products of the overexpressed genes is 15 inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the overexpressed gene product or gene products.

As noted above, antisense nucleic acids corresponding to the nucleotide sequence of PRC 1-88, 296-321, 458-537 can be used to reduce the expression level of the PRC 1-88, 20 296-321, 458-537. Antisense nucleic acids corresponding to PRC 1-88, 296-321, 458-537 that are up-regulated in either or both of PRC and PIN are useful for the treatment of either or both of PRC and PIN. Specifically, the antisense nucleic acids of the present invention may act by binding to the PRC 1-88, 296-321, 458-537 or mRNAs corresponding thereto, thereby inhibiting the transcription or translation of the genes, promoting the degradation 25 of the mRNAs, and/or inhibiting the expression of proteins encoded by a nucleic acid selected from the group consisting of the PRC 1-88, 296-321, 458-537, finally inhibiting the function of the proteins . The term "antisense nucleic acids" as used herein encompasses both nucleotides that are entirely complementary to the target sequence and those having a mismatch of one or more nucleotides, so long as the antisense nucleic acids 30 can specifically hybridize to the target sequences. For example, the antisense nucleic acids of the present invention include polynucleotides that have a homology of at least 70% or higher, preferably at 80% or higher, more preferably 90% or higher, even more preferably

95% or higher over a span of at least 15 continuous nucleotides. Algorithms known in the art can be used to determine the homology.

The antisense nucleic acid derivatives of the present invention act on cells producing the proteins encoded by marker genes by binding to the DNAs or mRNAs encoding the proteins, inhibiting their transcription or translation, promoting the degradation of the mRNAs, and inhibiting the expression of the proteins, thereby resulting in the inhibition of the protein function.

An antisense nucleic acid derivative of the present invention can be made into an external preparation, such as a liniment or a poultice, by mixing with a suitable base material which is inactive against the derivative.

Also, as needed, the derivatives can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, and such. These can be prepared by following known methods.

The antisense nucleic acids derivative is given to the patient by directly applying onto the ailing site or by injecting into a blood vessel so that it will reach the site of ailment. An antisense-mounting medium can also be used to increase durability and membrane-permeability. Examples are, liposomes, poly-L-lysine, lipids, cholesterol, lipofectin or derivatives of these.

The dosage of the antisense nucleic acid derivative of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

The antisense nucleic acids of the invention inhibit the expression of the protein of the invention and is thereby useful for suppressing the biological activity of a protein of the invention. Also, expression-inhibitors, comprising the antisense nucleic acids of the invention, are useful since they can inhibit the biological activity of a protein of the invention.

The antisense nucleic acids of present invention include modified oligonucleotides. For example, thioated nucleotides may be used to confer nuclease resistance to an oligonucleotide.

Also, a siRNA against marker gene can be used to reduce the expression level of the marker gene. By the term "siRNA" is meant a double stranded RNA molecule which

prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. In the context of the present invention, the siRNA comprises a sense nucleic acid sequence and an anti-sense nucleic acid sequence against an upregulated marker gene, such as PRC 5 1-88, 296-321, 458-537. The siRNA is constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, e.g., a hairpin.

The method is used to alter the expression in a cell of an upregulated, e.g., as a result of malignant transformation of the cells. Binding of the siRNA to a transcript corresponding to one of the PRC 1-88, 296-321, 458-537 in the target cell results in a 10 reduction in the protein production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally-occurring transcript. Preferably, the oligonucleotide is 19-25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50 , 25 nucleotides in length.

15 The nucleotide sequence of the siRNAs were designed using a siRNA design computer program available from the Ambion website ([http://www.ambion.com/techlib/mis](http://www.ambion.com/techlib/misc/siRNA_finder.html)c/siRNA_finder.html). The computer program selects nucleotide sequences for siRNA synthesis based on the following protocol.

Selection of siRNA Target Sites:

1. Beginning with the AUG start codon of the object transcript, scan downstream for AA 20 dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl, et al. recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with the 25 binding of the siRNA endonuclease complex.
2. Compare the potential target sites to the human genome database and eliminate from consideration any target sequences with significant homology to other coding sequences. The homology search can be performed using BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/
- 30 3. Select qualifying target sequences for synthesis. At Ambion, preferably several target sequences can be selected along the length of the gene for evaluation

The antisense oligonucleotide or siRNA of the invention inhibit the expression of the polypeptide of the invention and is thereby useful for suppressing the biological activity of the polypeptide of the invention. Also, expression-inhibitors, comprising the antisense oligonucleotide or siRNA of the invention, are useful in the point that they can inhibit the 5 biological activity of the polypeptide of the invention. Therefore, a composition comprising the antisense oligonucleotide or siRNA of the present invention are useful in treating a PRC or PIN.

Alternatively, function of one or more gene products of the over-expressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of 10 the gene products. For example, the compound is an antibody which binds to the over-expressed gene product or gene products.

The present invention refers to the use of antibodies, particularly antibodies against a protein encoded by an up-regulated marker gene, or a fragment of the antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule having a specific 15 structure, that interacts (i.e., binds) only with the antigen that was used for synthesizing the antibody (i.e., the up-regulated marker gene product) or with an antigen closely related to it. Furthermore, an antibody may be a fragment of an antibody or a modified antibody, so long as it binds to one or more of the proteins encoded by the marker genes. For instance, the antibody fragment may be Fab, F(ab')₂, Fv, or single chain Fv (scFv), in which Fv 20 fragments from H and L chains are ligated by an appropriate linker (Huston J. S. et al. Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an 25 expression vector, and expressed in an appropriate host cell (see, for example, Co M. S. et al. J. Immunol. 152:2968-2976 (1994); Better M. and Horwitz A. H. Methods Enzymol. 178:476-496 (1989); Pluckthun A. and Skerra A. Methods Enzymol. 178:497-515 (1989); Lamoyi E. Methods Enzymol. 121:652-663 (1986); Rousseaux J. et al. Methods Enzymol. 121:663-669 (1986); Bird R. E. and Walker B. W. Trends Biotechnol. 9:132-137 (1991)).

An antibody may be modified by conjugation with a variety of molecules, such as 30 polyethylene glycol (PEG). The present invention provides such modified antibodies. The modified antibody can be obtained by chemically modifying an antibody. These modification methods are conventional in the field.

Alternatively, an antibody may be obtained as a chimeric antibody, between a variable region derived from a nonhuman antibody and a constant region derived from a human antibody, or as a humanized antibody, comprising the complementarity determining region (CDR) derived from a nonhuman antibody, the frame work region (FR) derived 5 from a human antibody, and the constant region. Such antibodies can be prepared by using known technologies.

Cancer therapies directed at specific molecular alterations that occur in cancer cells have been validated through clinical development and regulatory approval of anti-cancer drugs such as trastuzumab (Herceptin) for the treatment of advanced breast cancer, 10 imatinib methylate (Gleevec) for chronic myeloid leukemia, gefitinib (Iressa) for non-small cell lung cancer (NSCLC), and rituximab (anti-CD20 mAb) for B-cell lymphoma and mantle cell lymphoma (Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res.* 2001 Oct;7(10):2958-70. Review.; Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, 15 Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med.* 2001 Mar 15;344(11):783-92.; Rehwald U, Schulz H, Reiser M, Sieber M, Staak JO, Morschhauser F, Driessen C, Rudiger T, Muller-Hermelink K, Diehl V, Engert A. Treatment of relapsed CD20+ Hodgkin lymphoma with the 20 monoclonal antibody rituximab is effective and well tolerated: results of a phase 2 trial of the German Hodgkin Lymphoma Study Group. *Blood.* 2003 Jan 15;101(2):420-424.; Fang G, Kim CN, Perkins CL, Ramadevi N, Winton E, Wittmann S and Bhalla KN. (2000). *Blood*, 96, 2246-2253.). These drugs are clinically effective and better tolerated than traditional anti-cancer agents because they target only transformed cells. Hence, such 25 drugs not only improve survival and quality of life for cancer patients, but also validate the concept of molecularly targeted cancer therapy. Furthermore, targeted drugs can enhance the efficacy of standard chemotherapy when used in combination with it (Gianni L. (2002). *Oncology*, 63 Suppl 1, 47-56.; Klejman A, Rushen L, Morrione A, Slupianek A and Skorski T. (2002). *Oncogene*, 21, 5868-5876.). Therefore, future cancer treatments will 30 probably involve combining conventional drugs with target-specific agents aimed at different characteristics of tumor cells such as angiogenesis and invasiveness.

These modulatory methods are performed *ex vivo* or *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). The method involves administering a protein or combination of proteins or a nucleic acid molecule or combination of nucleic acid, molecules as therapy to counteract aberrant expression or activity of the differentially expressed genes.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity of the genes may be treated with therapeutics that antagonize (i.e., reduce or inhibit) activity of the overexpressed gene or genes. Therapeutics that antagonize activity are administered therapeutically or prophylactically.

Therapeutics that may be utilized include, e.g., (i) a polypeptide, or analogs, derivatives, fragments or homologs thereof of the underexpressed gene or genes; (ii) antibodies to the overexpressed gene or genes; (iii) nucleic acids encoding the underexpressed gene or genes; (iv) antisense nucleic acids or nucleic acids that are "dysfunctional" (i.e., due to a heterologous insertion within the coding sequences of one or more overexpressed genes); (v) small interfering RNA (siRNA); or (vi) modulators (i.e., inhibitors, agonists and antagonists that alter the interaction between an over/underexpressed polypeptide and its binding partner. The dysfunctional antisense molecules are utilized to "knockout" endogenous function of a polypeptide by homologous recombination (see, e.g., Capecchi, *Science* 244: 1288-1292 1989)

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with therapeutics that increase (i.e., are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a polypeptide (or analogs, derivatives, fragments or homologs thereof) or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (e.g., from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a gene whose expression is altered). Methods that are well-known within the art include, but are not limited to, immunoassays (e.g., by Western blot analysis, immunoprecipitation

followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (e.g., Northern assays, dot blots, *in situ* hybridization, etc.).

5 Prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Therapeutic methods include contacting a cell with an agent that modulates one or more of the activities of the gene products of the differentially expressed genes. An agent that modulates protein activity includes a nucleic acid or a protein, a naturally-occurring 10 cognate ligand of these proteins, a peptide, a peptidomimetic, or other small molecule. For example, the agent stimulates one or more protein activities of one or more of a differentially under-expressed gene.

The present invention also relates to a method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a vaccine 15 comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide or the fragment thereof. An administration of the polypeptide induce an anti-tumor immunity in a subject. To inducing anti-tumor immunity, a polypeptide encoded by a nucleic acid selected from the group consisting of 20 PRC 1-88, 296-321, 458-537 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide is administered. The polypeptide or the immunologically active fragments thereof are useful as vaccines against either or both of PRC and PIN. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell receptor (TCR) or presented by an antigen presenting cell (APC), 25 such as macrophage, dendritic cell (DC), or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

In the present invention, vaccine against either or both of PRC and PIN refers to a substance that has the function to induce anti-tumor immunity upon inoculation into animals. According to the present invention, polypeptides encoded by a nucleic acid 30 selected from the group consisting of PRC 1-88, 296-321, 458-537 or fragments thereof were suggested to be HLA-A24 or HLA-A*0201 restricted epitopes peptides that may induce potent and specific immune response against either or both of PRC and PIN cells

expressing PRC 1-88, 296-321, 458-537. Thus, the present invention also encompasses method of inducing anti-tumor immunity using the polypeptides. In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors,
- 5 - induction of antibodies that recognize tumors, and
- induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by 10 observing in vivo or in vitro the response of the immune system in the host against the protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen 15 presented by APC in antigen specific manner differentiate into cytotoxic T cells (or cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells). Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to T cell by APC, and detecting the induction of CTL. Furthermore, APC has the effect of activating CD4+ T cells, CD8+ T cells, 20 macrophages, eosinophils, and NK cells. Since CD4+ T cells and CD8+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL 25 inducing action among APCs. In this method, the test polypeptide is initially contacted with DC, and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ^{51}Cr -labeled tumor cells as the indicator. 30 Alternatively, the method of evaluating the degree of tumor cell damage using ^3H -thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator is also well known.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported that it can be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

5 The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against tumors. Furthermore, APC that acquired the ability to induce CTL against tumors by contacting with the polypeptides are useful as vaccines against tumors.

10 Furthermore, CTL that acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against tumors. Such therapeutic methods for tumors using anti-tumor immunity due to APC and CTL are referred to as cellular immunotherapy.

15 Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction is known to increase by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

20 Alternatively, the induction of anti-tumor immunity by a polypeptide can be confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth of tumor cells is suppressed by those antibodies, the polypeptide can be determined to have an ability to induce anti-tumor immunity.

25 Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of either or both of PRC and PIN. Therapy against cancer or prevention of the onset of cancer includes any of the steps, such as inhibition of the growth of cancerous cells, involution of cancer, and suppression of occurrence of cancer. Decrease in mortality of individuals having cancer, decrease of tumor markers in the blood, alleviation of detectable symptoms accompanying cancer, and such are also included in the therapy or prevention of cancer.

30 Such therapeutic and preventive effects are preferably statistically significant. For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against cell proliferative diseases is compared to a control

without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test, or ANOVA may be used for statistical analyses.

The above-mentioned protein having immunological activity or a vector encoding the protein may be combined with an adjuvant. An adjuvant refers to a compound that 5 enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Examples of adjuvants include cholera toxin, salmonella toxin, alum, and such, but are not limited thereto. Furthermore, the vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, 10 physiological saline, phosphate buffer, culture fluid, and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants, and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration, or boosted by multiple administrations.

When using APC or CTL as the vaccine of this invention, tumors can be treated or 15 prevented, for example, by the ex vivo method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, the cells are contacted with the polypeptide ex vivo, and following the induction of APC or CTL, the cells may be administered to the subject. APC can be also induced by introducing a vector encoding the polypeptide into PBMCs ex vivo. APC or CTL induced in vitro can be cloned prior to administration. By 20 cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of tumors from other individuals.

Furthermore, a pharmaceutical composition for treating or preventing a cell 25 proliferative disease, such as cancer, comprising a pharmaceutically effective amount of the polypeptide of the present invention is provided. The pharmaceutical composition may be used for raising anti tumor immunity.

Pharmaceutical compositions for inhibiting PRC or PIN

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical 30 (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or

insufflation. Preferably, administration is intravenous. The formulations are optionally packaged in discrete dosage units

Pharmaceutical formulations suitable for oral administration include capsules, cachets or tablets, each containing a predetermined amount of the active ingredient.

- 5 Formulations also include powders, granules or solutions, suspensions or emulsions. The active ingredient is optionally administered as a bolus electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients.
- 10 Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The
- 15 20 tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein. A package of tablets may contain one tablet to be taken on each day of the month.

- 25 Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions
- 30

may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration include suppositories with standard carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the 5 mouth, for example buccally or sublingually, include lozenges, which contain the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intra-nasal administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with 10 an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents.

For administration by inhalation the compounds are conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as 15 dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds may take the form of a dry powder composition, for example a powder mix of the compound 20 and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflators.

Other formulations include implantable devices and adhesive patches; which release a therapeutic agent.

25 When desired, the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

It should be understood that in addition to the ingredients particularly mentioned 30 above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

For each of the aforementioned conditions, the compositions, e.g., polypeptides and organic compounds are administered orally or via injection at a dose of from about 0.1 to 5 about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually 10 from about 100 mg to about 500 mg.

The dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

The invention will be further described in the following examples, which do not 15 limit the scope of the invention described in the claims. The following examples illustrate the identification and characterization of genes differentially expressed in PRC or PIN cells.

EXAMPLE 1: PREPARATION OF TEST SAMPLES

Tissue obtained from diseased tissue (e.g., epithelial cells from PRCs) and normal 20 tissues was evaluated to identify genes which are differently expressed or a disease state, e.g., PRC. The assays were carried out as follows.

Patients, tissue samples and Laser-capture microdissection (LCM)

PRC samples including non-cancerous prostate tissues were obtained from 26 25 patients who underwent radical prostatectomy without preoperative treatment. Prostate adenocarcinomas or high-grade PINs were histopathologically diagnosed by a single pathologist (M.F.). Among 26 PRC tissues, 20 cancers and 10 high-grade PINs cells that have sufficient amount and quality of RNA to analyze were used for microarray study. Clinical and pathological information on the tumor is detailed in Table 1. 30 Samples were embedded in TissueTek OCT medium (Sakura) and then stored at -80°C until use. Frozen specimens were serially sectioned in 8-μm slices with a cryostat and

stained with hematoxylin and eosin to define the analyzed regions. To avoid cross-contamination of cancer and noncancerous cells, the two populations were prepared by EZ Cut LCM System (SL Microtest GmbH) following the manufacture's protocol with several modifications.

5

Table 1 Clinicopathological features

Case	Age	PSA (ng/ml)	Pathological Stage	Microdissected Lesions
1	76	17.0	pT2aN0M0	T ^(a)
2	73	14.0	pT2aN0M0	T
3	73	59.2	pT3aN0M0	T
4	56	8.6	pT2bN0M0	T
5	73	1.8	pT2aN0M0	PIN
6	61	8.9	pT2bN0M0	T
7	71	11.4	pT2bN0M0	T
8	69	9.5	pT2aN0M0	PIN
9	66	9.6	pT3aN0M0	T
10	62	6.7	pT2aN0M0	PIN
11	56	35.0	pT3bN0M0	PIN
12	66	12.0	pT2bN0M0	T
13	65	4.1	pT2bN0M0	T
14	77	12.3	pT2bN0M0	T, PIN
15	69	10.4	pT2bN0M0	T
16	68	14.1	pT3aN0M0	T, PIN
17	NA ^(b)	10.5	pT2bN0M0	T
18	NA	NA	NA	T
19	63	4.5	pT2bN0M0	T
20	67	9.8	pT3aN0M0	T
21	63	12.4	pT3N0M0	T
22	73	13.0	pT3bN1M0	T
23	75	10.0	pT2aN1M0	T, PIN
24	67	3.3	pT3aN0M0	T, PIN
25	64	5.7	pT2bN0M0	PIN
26	69	38.0	pT3aN0M0	PIN

^(a)T indicates prostate cancer. ^(b)NA: not available

Extraction of RNA and T7-based RNA amplification

Total RNA was extracted from each population of laser captured cells into 350µl RLT lysis buffer (QIAGEN). The extracted RNA was treated for 30 minutes at

room temperature with 30 units of DNase I (QIAGEN) in the presence of 1 unit of RNase inhibitor (TOYOBO, Osaka, Japan) to eliminate any contaminating genomic DNA. After inactivation at 70°C for 10 min, the RNAs were purified with an RNeasy Mini Kit (QIAGEN) according to the manufacturer's recommendations and DNase-treated RNAs were subjected to T7-based RNA amplification. Two rounds of amplification yielded 50-100 µg of amplified RNA (aRNA) for each sample. 2.5µg aliquots of aRNA from each cancerous cell and noncancerous cell were reverse-transcribed in the presence of Cy5-dCTP and Cy3-dCTP, respectively.

Preparation of the cDNA microarray

10 A "genome-wide" cDNA microarray system was prepared containing 23,040 cDNAs selected from the UniGene database o (build #131) the National Center for Biotechnology Information (NCBI). Briefly, the cDNAs were amplified by reverse transcription-PCR using poly(A)+RNA isolated from various human organs as templates; lengths of the amplicons ranged from 200 to 1100 bp without repetitive or 15 poly(A) sequences. The PCR products were spotted in duplicate on type-7 glass slides (Amersham Bioscience) using an Array Spotter Generation III (Amersham Bioscience). Each slide contained 52 housekeeping genes, to normalize the signal intensities of the different fluorescent dyes.

Hybridization and acquisition of data

20 Hybridization and washing were performed according to protocols described previously except that all processes were carried out with an Automated Slide Processor (Amersham Biosciences) (17). The intensity of each hybridization signal was calculated photometrically by the ArrayVision computer program (Amersham Biosciences) and background intensity was subtracted. Normalization of each Cy3- and Cy5 signal intensity 25 was performed using averaged signals from the 52 housekeeping genes. A cut-off value for each expression level was automatically calculated according to background fluctuation. When both Cy3 and Cy5 signal intensities were lower than the cut-off values, expression of the corresponding gene in that sample was assessed as absent. The Cy5/Cy3 ratio was calculated as the relative expression ratio. For other genes we calculated the Cy5/Cy3 ratio 30 using raw data of each sample.

EXAMPLE 2 : IDENTIFICATION OF PRC – ASSOCIATED GENES

When up- or down-regulated genes common to PRC and PINs were identified, the genes were analyzed by the following criteria. Initially, genes whose relative expression ratio was able to be calculated for more than 50% cases and whose expression were up- or down-regulated in more than 50% of cases were selected. The relative expression ratio of each gene (Cy5/Cy3 intensity ratio) was classified into one of four categories: (1) up-regulated (expression ratio more than 3.0 in more than 50% of the informative; (2) down-regulated (expression ratio less than 0.33 in more than 50% of the informative cases; (3) unchanged expression (expression ratio between 0.33 and 3.0 in more than 50% of the informative cases); and (4) not expressed (or slight expression but under the cut-off level for detection). These categories were defined to detect a set of genes whose changes in expression ratios were common among samples as well as specific to a certain subgroup. To detect candidate genes that were commonly up- or down-regulated in either or both of PRC and PIN cell, the overall expression patterns of 23,040 genes were screened to select genes with expression ratios of more than 3.0 or less than 0.33 that were present in more than 50% of the PRC cases categorized as (1), (2), or (3).

Furthermore when up- or down-regulated genes common to PRC or PINs were identified, the genes were analyzed by the following criteria. Initially, genes whose relative expression ratio was able to be calculated for more than 50% cases and whose expression were up- or down-regulated in more than 50% of cases were selected. The relative expression ratio of each gene (Cy5/Cy3 intensity ratio) was classified into one of four categories: (5) up-regulated (expression ratio more than 5.0 in more than 50% of the informative; (6) down-regulated (expression ratio less than 0.2 in more than 50% of the informative cases; (7) unchanged expression (expression ratio between 0.2 and 5.0 in more than 50% of the informative cases); and (8) not expressed (or slight expression but under the cut-off level for detection). These categories were defined to detect a set of genes whose changes in expression ratios were common among samples as well as specific to a certain subgroup. To detect candidate genes that were commonly up- or down-regulated in either or both of PRC and PIN cell, the overall expression patterns of 23,040 genes were screened to select genes with expression ratios of more than 5.0 or less than 0.2 that were present in more than 50% of the PRC cases categorized as (5), (6), or (7).

Identification of genes with clinically relevant expression patterns in PRC cells

The expression patterns of approximately 23,000 genes were investigated in PRC cells using cDNA microarray. Individual data was excluded when both Cy5 and Cy3 signals were under cut-off values. 88 up-regulated genes were identified whose expression ratio was more than 3.0 in PRC and PINs (see Table 3), whereas 207 down-regulated genes whose expression ratio was less than 0.33 were identified (see Table 4). 26 up-regulated genes were identified whose expression ratio was more than 5.0 in PRC (see Table 5), whereas 136 down-regulated genes whose expression ratio was less than 0.2 were identified (see Table 6).

10 Among the up-regulated genes, α -methylacyl coenzyme A racemase (AMACR) has been already reported to be overexpressed in PRC (13). Furthermore, these up-regulated elements included significant genes involved in metabolism and signal transduction pathway, transcriptional factors, cell cycle, oncogene, and cell adhesion and cytoskeleton. Of them, olfactory receptor, family 51, subfamily E, member 2 (*OR51E2*) 15 that is prostate specific G-protein coupled receptor (*PSGR*), and PRC overexpressed gene 1 (*POV1*) had already been reported as over-expressed in PRCs (Luo et al., 2002; Cole et al., 1998; Xu et al., 2000) (see Table 5).

20 80 up-regulated genes were identified whose expression ratio was more than 5.0 in PINs (see Table 7), whereas 155 down-regulated genes whose expression ratio was less than 0.2 were identified (see Table 8).

To confirm the reliability of the expression indicated by microarray analysis, semi-quantitative RT-PCR experiments were performed. Four up-regulated genes were selected and their expression levels measured by semi-quantitative RT-PCR. A 3- μ g aliquot of aRNA from each sample was reverse-transcribed for single-stranded cDNAs 25 using random primer (Roche) and Superscript II (Life Technologies, Inc.). Each cDNA mixture was diluted for subsequent PCR amplification with the primer sets that were shown in Table 2. Expression of β -actin (ACTB) served as an internal control. PCR reactions were optimized for the number of cycles to ensure product intensity within the linear phase of amplification.

30 Comparing the ratios of the expression levels of the 4 up-regulated genes (AMACR, HOXC6, POV1, ABHD2 and C20ORF102) whose expression were overexpressed in almost of all informative cases the results were highly similar to those of

the microarray analysis in the great majority of the tested cases (Fig. 1). These data verified the reliability of our strategy to identify commonly up-regulated genes in PRC cells.

Table 3 Commonly up-regulated genes in prostate cancers and PINs

PRC Assi gnm ent	Accession No.	Hs. 76893	Symbol BDH	Title
function known				
1	M93107	76893	BDH	3-hydroxybutyrate dehydrogenase (heart, mitochondrial)
2	U89281	11958	RODH	3-hydroxysteroid epimerase
3	L41559	3192	PCBD	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1)
4	AJ130733	128749	AMACR	alpha-methylacyl-CoA racemase
5	S77410	89472	AGTR1	angiotensin II receptor, type 1
6	AI080640	413945	AGR2	anterior gradient 2 homolog (Xenopus laevis)
7	NM_00048	88251	ARSA	arylsulfatase A
8	AF071202	139336	ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
9	NM_00006	78885	BTD	biotinidase
10	D90276	12	CEACAM4	carcinoembryonic antigen-related cell adhesion molecule 4
11	AB030905	406384	CBX3	chromobox homolog 3 (HP1 gamma homolog, Drosophila)
12	BF106962	20415	FAM3B	chromosome 21 open reading frame 11
13	AI817172	29423	COLEC12	collectin sub-family member 12
14	NM_00543	288862	D10S170	DNA segment on chromosome 10 (unique) 170
15	U31556	2331	E2F5	E2F transcription factor 5, p130-binding
16	AF039918	80975	ENTPD5	ectonucleoside triphosphate diphosphohydrolase 5
17	L10340	2642	EEF1A2	eukaryotic translation elongation factor 1 alpha 2
18	AI984005	380785	XPOT	exportin, tRNA (nuclear export receptor)

				for tRNAs)
19	NM_00016	333303	GJB1	gap junction protein, beta 1, 32kDa (connexin 32, Charcot-Marie-Tooth neuropathy, X-linked)
6				
20	AF040260	105435	GMDS	GDP-mannose 4,6-dehydratase
21	AF236056	182793	GOLPH2	golgi phosphoprotein 2
22	AF055013	203862	GNAI1	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1
23	NM_00085	75295	GUCY1A3	guanylate cyclase 1, soluble, alpha 3
6				
24	S82986	820	HOXC6	homeo box C6
25	U42408	18141	LAD1	ladinin 1
26	M88468	130607	MVK	mevalonate kinase (mevalonic aciduria)
27	D56064	167	MAP2	microtubule-associated protein 2
28	AI302799	68583	MIPEP	mitochondrial intermediate peptidase
29	AB002387	118483	MYO6	myosin VI
30	R22536	220324	FLJ13052	NAD kinase
31	AI246554	31547	NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa
32	AA858162	124673	NCAG1	NCAG1
33	AI805082	303171	OR51E2	olfactory receptor, family 51, subfamily E, member 2
34	U79240	79337	PASK	PAS domain containing serine/threonine kinase
35	BF690393	83383	PRDX4	peroxiredoxin 4
36	AK025460	286049	PSA	phosphoserine aminotransferase
37	NM_02120	380812	PLEKHB1	pleckstrin homology domain containing, family B (ejectins) member 1
0				
38	L14778	272458	PPP3CA	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha)
39	AF044588	344037	PRC1	protein regulator of cytokinesis 1
40	NM_00676	71119	N33	Putative prostate cancer tumor suppressor
5				
41	NM_01234	78776	NMA	putative transmembrane protein
2				
42	M77836	79217	PYCR1	pyrroline-5-carboxylate reductase 1
43	D42063	199179	RANBP2	RAN binding protein 2
44	AF064824	103755	RIPK2	receptor-interacting serine-threonine kinase 2

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45	N78357	302136	RIMS1	regulating synaptic membrane exocytosis 1
46	L10333	99947	RTN1	reticulon 1
47	Y18418	272822	RUVBL1	RuvB-like 1 (E. coli)
48	U80456	27311	SIM2	single-minded homolog 2 (Drosophila)
49	AF269150	8203	SMBP	SM-11044 binding protein
50	U17566	84190	SLC19A1	solute carrier family 19 (folate transporter), member 1
51	D88308	11729	SLC27A2	solute carrier family 27 (fatty acid transporter), member 2
52	AF007216	5462	SLC4A4	solute carrier family 4, sodium bicarbonate cotransporter, member 4
53	AD001528	89718	SMS	spermine synthase
54	M32313	552	SRD5A1	steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)
55	L15203	82961	TFF3	trefoil factor 3 (intestinal)
56	M91670	174070	E2-EPF	ubiquitin carrier protein
57	AW135763	6375	HT010	uncharacterized hypothalamus protein HT010
function unknown				
58	AA206763	7991	C20orf 102	chromosome 20 open reading frame 102
59	AI989530	240845	DKFZP 434D14	DKFZP434D146 protein
			6	
60	AI192351	76285	DKFZP 564B16	DKFZP564B167 protein
			7	
61	AI133467	95612		ESTs
62	H17800	438858		ESTs
63	AI732103			ESTs
64	AI671006	5794		ESTs, Moderately similar to hypothetical protein FLJ20234 [Homo sapiens] [H.sapiens]
65	AA420675	188826		ESTs, Moderately similar to RL39_HUMAN 60S ribosomal protein L39 [H.sapiens]
66	AI700341	110406		ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]

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67	BF057183	355809		ESTs, Weakly similar to male-specific lethal 3-like 1 isoform
68	H05758	355684		ESTs, Weakly similar to neuronal thread protein [Homo sapiens]
69	AA743348	120591		Homo sapiens cDNA FLJ35632 fis, clone SPLEN2011678.
70	AA679304	5740		Homo sapiens cDNA FLJ40165 fis, clone TESTI2015962.
71	AK027019	381105		Homo sapiens cDNA: FLJ23366 fis, clone HEP15665.
72	AA994004	128790		Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 1628928
73	H09779	283851		Homo sapiens mRNA; cDNA DKFZp547G036 (from clone DKFZp547G036)
74	BE254330	14846		Homo sapiens mRNA; cDNA DKFZp564D016 (from clone DKFZp564D016)
75	AL157505	21380		Homo sapiens mRNA; cDNA DKFZp586P1124 (from clone DKFZp586P1124)
76	AI217963	434541		Homo sapiens, clone IMAGE:4429946, mRNA
77	BF724600	22247		Homo sapiens, clone IMAGE:5302158, mRNA
78	NM_012066	128702	20D7- FC4	hypothetical protein 20D7-FC4
79	AB029008	84045	FLJ202 88	FLJ20288 protein
80	AK026325	235873	FLJ226 72	hypothetical protein FLJ22672
81	R55332	379386	LOC11 5286	hypothetical protein LOC115286
82	H12084	31110	MGC3 4827	hypothetical protein MGC34827
83	D29954	13421	KIAA0 056	KIAA0056 protein
84	AB020637	167115	KIAA0 830	KIAA0830 protein
85	AB023157	131945	KIAA0 940	KIAA0940 protein

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86	AB032981	102657	KIAA1 155	KIAA1155 protein
87	AB032983	21894	KIAA1 157	KIAA1157 protein
88	AB033091	446390	KIAA1 265	KIAA1265 protein

Table 4 Commonly down-regulated genes in prostate cancers and PINs

PRC Assi gnm ent	Accession No.	Hs.	Symbol	Title
function known				
89	NM_00252 6	153952	NTSE	5'-nucleotidase, ecto (CD73)
90	NM_00515 9	118127	ACTC	actin, alpha, cardiac muscle
91	NM_00161 5	378774	ACTG2	actin, gamma 2, smooth muscle, enteric
92	AL117643	99954	ACVR1B	activin A receptor, type IB
93	BG105547	324470	ADD3	adducin 3 (gamma)
94	AF245505	72157 922	DKFZp564I1	adlican
95	N74230	193228	AGXT2	alanine-glyoxylate aminotransferase 2
96	K03000	76392	ALDH1A1	aldehyde dehydrogenase 1 family, member A1
97	M28443	300280	AMY2A	amylase, alpha 2A; pancreatic
98	AF286598	9271	AMOT	angiomotin
99	NM_00070 0	78225	ANXA1	annexin A1
100	D00017	217493	ANXA2	annexin A2
101	NM_00115 5	118796	ANXA6	annexin A6
102	AK027126	160786	ASS	argininosuccinate synthetase
103	AA054346	32168	AUTS2	autism susceptibility candidate 2
104	AL117565	6607	AXUD1	AXIN1 up-regulated 1
105	AB004066	171825	BHLHB2	basic helix-loop-helix domain containing, class B, 2
106	M14745	79241	BCL2	B-cell CLL/lymphoma 2
107	S67310	69771	BF	B-factor, properdin
108	M69225	198689	BPAG1	bullous pemphigoid antigen 1,

				230/240kDa
109	X63629	2877	CDH3	cadherin 3, type 1, P-cadherin (placental)
110	R45979	252387	CELSR1	cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, <i>Drosophila</i>)
111	AF134640	7235	CACNG3	calcium channel, voltage-dependent, gamma subunit 3
112	D17408	21223	CNN1	calponin 1, basic, smooth muscle
113	K01144	84298	CD74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)
114	NM_00187 8	183650	CRABP2	cellular retinoic acid binding protein 2
115	NM_00299 6	80420	CX3CL1	chemokine (C-X3-C motif) ligand 1
116	U16306	81800	CSPG2	chondroitin sulfate proteoglycan 2 (versican)
117	AV648364	356416	CBX7	chromobox homolog 7
118	AF000959	110903	CLDN5	claudin 5 (transmembrane protein deleted in velocardiofacial syndrome)
119	NM_00183 1	75106	CLU	clusterin (SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2)
120	L02870	1640	COL7A1	collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
121	AF018081	78409	COL18A1	collagen, type XVIII, alpha 1
122	NM_00173 3	1279	C1R	complement component 1, r subcomponent
123	J04080	434029	C1S	complement component 1, s subcomponent
124	K02765	284394	C3	complement component 3
125	AF007162	408767	CRYAB	crystallin, alpha B
126	L12579	147049	CUTL1	cut-like 1, CCAAT displacement protein (<i>Drosophila</i>)
127	NM_00007 6	106070	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)
128	BF183952	412999	CSTA	cystatin A (stefin A)
129	NM_00407 8	108080	CSRP1	cysteine and glycine-rich protein 1
130	J04813	104117	CYP3A5	cytochrome P450, family 3, subfamily A,

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				polypeptide 5
131	AF070590	90869	LOC90957	DEAH-box RNA/DNA helicase AAM73547
132	NM_00439 4	75189	DAP	death-associated protein
133	D83407	156007	DSCR1L1	Down syndrome critical region gene 1-like 1
134	L11329	1183	DUSP2	dual specificity phosphatase 2
135	AW002941	339283	ERAP140	endoplasmic reticulum associated protein 140 kDa
136	J04162	176663	FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor for (CD16)
137	M87770	278581	FGFR2	fibroblast growth factor receptor 2
138	X02761	287820	FN1	fibronectin 1
139	NM_00145 6	195464	FLNA	filamin A, alpha (actin binding protein 280)
140	U60115	239069	FHL1	four and a half LIM domains 1
141	L42176	8302	FHL2	four and a half LIM domains 2
142	U28963	380901	GPS2	G protein pathway suppressor 2
143	AW949747	169946	GATA3	GATA binding protein 3
144	AK021685	234896	GMNN	geminin, DNA replication inhibitor
145	BF115308	132760	G6PT1	glucose-6-phosphatase, transport (glucose-6-phosphate) protein 1
146	NM_00208 3	2704	GPX2	glutathione peroxidase 2 (gastrointestinal)
147	NM_00208 4	386793	GPX3	glutathione peroxidase 3 (plasma)
148	AA290738	301961	GSTM1	glutathione S-transferase M1
149	NM_00208 1	2699	GPC1	glypican 1
150	M55543	171862	GBP2	guanylate binding protein 2, interferon-inducible
151	NM_00018 6	250651	HF1	H factor 1 (complement)
152	AK000415	250666	HES1	hairy and enhancer of split 1, (<i>Drosophila</i>)
153	AA522530	111244	RTP801	HIF-1 responsive RTP801
154	AA490691	421136	HOXD11	homeo box D11
155	J02770	36602	IF	I factor (complement)
156	S81914	76095	IER3	immediate early response 3
157	M87790	102950	IGLJ3	immunoglobulin lambda joining 3

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158	L08488	32309	INPP1	inositol polyphosphate-1-phosphatase
159	M31159	77326	IGFBP3	insulin-like growth factor binding protein 3
160	M59911	265829	ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
161	X52186	85266	ITGB4	integrin, beta 4
162	NM_00643	174195	IFITM2	interferon induced transmembrane protein 2 (1-8D)
163	NM_00219	80645	IRF1	interferon regulatory factor 1
	8			
164	AF020201	166154	JAG2	jagged 2
165	M25629	123107	KLK1	kallikrein 1, renal/pancreas/salivary
166	X14640	74070	KRT13	keratin 13
167	X07696	80342	KRT15	keratin 15
168	NM_00042	2785	KRT17	keratin 17
	2			
169	Y00503	182265	KRT19	keratin 19
170	M21389	433845	KRT5	keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)
171	X03212	23881	KRT7	keratin 7
172	NM_00022	2783	KRT9	keratin 9 (epidermolytic palmoplantar keratoderma)
173	D14520	84728	KLF5	Kruppel-like factor 5 (intestinal)
174	U07643	105938	LTF	lactotransferrin
175	D37766	75517	LAMB3	laminin, beta 3
176	AW139663	166254	VMP1	likely ortholog of rat vacuole membrane protein 1
177	AF002672	152944	LOH11CR2A	loss of heterozygosity, 11, chromosomal region 2, gene A
178	AI814306	42438	LSM6	LSM6 homolog, U6 small nuclear RNA associated (<i>S. cerevisiae</i>)
179	Z68179	77667	LY6E	lymphocyte antigen 6 complex, locus E
180	BE621666	296398	LAPTM4B	lysosomal associated protein transmembrane 4 beta
181	M33906	198253	HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1
182	K01171	409805	HLA-DRA	major histocompatibility complex, class II, DR alpha
183	M15178	318720	HLA-DRB4	major histocompatibility complex, class II, DR beta 4

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184	BF697545	365706	MGP	matrix Gla protein
185	AW298180	2256	MMP7	matrix metalloproteinase 7 (matrilysin, uterine)
186	AF017418	104105	MEIS2	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse)
187	BF971884	118786	MT2A	metallothionein 2A
188	NM_00592	3745	MFGE8	milk fat globule-EGF factor 8 protein
189	J05581	89603	MUC1	mucin 1, transmembrane
190	AA628530	405873	ISYNA1	myo-inositol 1-phosphate synthase A1
191	J02854	9615	MYL9	myosin, light polypeptide 9, regulatory neighbor of COX4
192	AF005888	173162	NOC4	neuropilin 1
193	AA886412	69285	NRP1	nuclear factor I/X (CCAAT-binding transcription factor)
194	L31881	35841	NFIX	nuclear receptor subfamily 4, group A, member 2
195	X75918	82120	NR4A2	orosomucoid 1
196	M13692	572	ORM1	PDZ and LIM domain 1 (elfin)
197	U90878	75807	PDLIM1	peroxisome proliferative activated receptor, alpha
198	NM_00503	998	PPARA	phosphatidic acid phosphatase type 2C
199	AF035959	24879	PPAP2C	phosphatidylinositol glycan, class Q
200	AB003723	18079	PIGQ	plasminogen activator, urokinase
201	D00244	77274	PLAU	platelet derived growth factor C
202	AF091434	43080	PDGFC	prominin-like 1 (mouse)
203	AF027208	112360	PROML1	prostaglandin D2 synthase 21kDa (brain)
204	AL045876	430637	PTGDS	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9
205	BF510741	5648	PSMD9	protein kinase, cAMP-dependent, regulatory, type I, beta
206	M65066	1519	PRKAR1B	ras homolog gene family, member F (in filopodia)
207	AW249758	96593	ARHF	regulator of G-protein signalling 1
208	X73427	75256	RGS1	retinoblastoma binding protein 8
209	U72066	29287	RBBP8	retinoic acid induced 3
210	NM_00397	194691	RAI3	Rho GDP dissociation inhibitor (GDI) beta
211	L20688	83656	ARHGDI	RNA binding motif, single stranded interacting protein 1
212	X64652	241567	RBMS1	

213	AA173755	301198	ROBO1	roundabout, axon guidance receptor, homolog 1 (<i>Drosophila</i>)
214	AF132734	107394	SEC8	secretory protein SEC8
215	NM_00463	82222	SEMA3B	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B
216	M93056	183583	SERPINB1	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1
217	M13690	151242	SERPING1	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1
218	NM_00645	288215	STHM	sialyltransferase
219	AF070609	75379	SLC1A3	solute carrier family 1 (glial high affinity glutamate transporter), member 3
220	AF215636	5944	SLC11A3	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 3
221	U59299	90911	SLC16A5	solute carrier family 16 (monocarboxylic acid transporters), member 5
222	Y08110	101657	SORL1	sortilin-related receptor, L(DLR class) A repeats-containing
223	M81635	160483	STOM	stomatin
224	U15131	79265	ST5	suppression of tumorigenicity 5
225	BF514189	345728	SOCS3	suppressor of cytokine signaling 3
226	AI423028	71622	SMARCD3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3
227	U21847	82173	TIEG	TGFB inducible early growth response
228	U54831	75248	TOP2B	topoisomerase (DNA) II beta 180kDa
229	S95936	396489	TF	transferrin
230	M77349	118787	TGFBI	transforming growth factor, beta-induced, 68kDa
231	NM_00318	433399	TAGLN	transgelin
232	6			
232	M98479	75307	TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
233	L24203	82237	TRIM29	tripartite motif-containing 29
234	AF208860	159651	TNFRSF21	tumor necrosis factor receptor superfamily, member 21
235	U44839	171501	USP11	ubiquitin specific protease 11

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236	L13852	16695	UBE1L	ubiquitin-activating enzyme E1-like
237	X63187	2719	WFDC2	WAP four-disulfide core domain 2
238	AF122922	284122	WIF1	WNT inhibitory factor 1
239	AA909999	50216	ZFD25	zinc finger protein (ZFD25)
240	AA916688	85155	ZFP36L1	zinc finger protein 36, C3H type-like 1
			function unknown	
241	AB002384	101359	C6orf32	chromosome 6 open reading frame 32
242	AA620628	186486		ESTs
243	AA632025	444752		ESTs
244	AA904658	117299		ESTs
245	AI022658	292171		ESTs
246	AI027791	132296		ESTs
247	AI338011	132147		ESTs
248	AI732637	277901		ESTs
249	BE868254	380149		ESTs
250	H53099	420009		ESTs
251	N95414	55168		ESTs, Weakly similar to neuronal thread protein [Homo sapiens] [H.sapiens]
252	BG163478	405950		ESTs, Weakly similar to BAI1_HUMAN Brain-specific angiogenesis inhibitor 1 precursor [H.sapiens]
253	AI342255	24192		Homo sapiens cDNA FLJ20767 fis, clone COL06986.
254	AI651212	4283		Homo sapiens cDNA FLJ31125 fis, clone IMR322000819.
255	AW967916	31944		Homo sapiens cDNA FLJ33236 fis, clone ASTRO2002571.
256	AI566720	380045		Homo sapiens cDNA FLJ34528 fis, clone HLUNG2008066
257	W93000	59389		Homo sapiens cDNA FLJ38601 fis, clone HEART2003781.
258	BE885999	397414		Homo sapiens cDNA: FLJ20860 fis, clone ADKA01632.
259	AK025909	288741		Homo sapiens cDNA: FLJ22256 fis, clone HRC02860.
260	AK025953	380437		Homo sapiens cDNA: FLJ22300 fis, clone HRC04759.
261	BF311166	110783		Homo sapiens cDNA: FLJ22365 fis, clone HRC06613.
262	AI097529	8136		Homo sapiens clone 23698 mRNA sequence

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263	AI269367	101307		Homo sapiens HUT11 protein mRNA, partial 3' UTR
264	N58556	323053		Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 26539.
265	AL110236	321022		Homo sapiens mRNA; cDNA DKFZp566P1124 (from clone DKFZp566P1124)
266	BF791544	351680		Homo sapiens, clone IMAGE:4103364, mRNA
267	AV733210	367688		Homo sapiens, clone IMAGE:4794726, mRNA
268	AA456955	78026		Homo sapiens, Similar to hypothetical protein C130031J23, clone IMAGE:3445545, mRNA, partial cds
269	AL120399	343567	LOC151568	hypothetical protein BC009491
270	BE539165	355793	DKFZp313M 0720	hypothetical protein DKFZp313M0720
271	AA709155	104800	FLJ10134	hypothetical protein FLJ10134
272	AK001061	30925	FLJ10199	hypothetical protein FLJ10199
273	AK001431	5105	FLJ10569	hypothetical protein FLJ10569
274	AI709055	115412	FLJ13881	hypothetical protein FLJ13881
275	AK026058	27556	FLJ22405	hypothetical protein FLJ22405
276	AW271223	5890	FLJ23306	hypothetical protein FLJ23306
277	N31935	220745	FLJ25604	hypothetical protein FLJ25604
278	AK001839	206501	LOC57228	hypothetical protein from clone 643
279	AK022547	8694	LOC56965	hypothetical protein from EUROIMAGE 1977056
280	AA180145	351270	LOC152485	hypothetical protein LOC152485
281	AK024828	69388	LOC221749	hypothetical protein LOC221749
282	AV758898	366	MGC27165	hypothetical protein MGC27165
283	AW888223	59384	MGC3047	hypothetical protein MGC3047
284	AA133590	377830	MGC44669	hypothetical protein MGC44669
285	L13720	207251	MGC5560	hypothetical protein MGC5560
286	AI206046	50535	MGC7036	hypothetical protein MGC7036
287	AB002319	8663	KIAA0321	KIAA0321 protein
288	AB011125	105749	KIAA0553	KIAA0553 protein
289	AB037797	24684	KIAA1376	KIAA1376 protein
290	AI741882	278436	KIAA1474	KIAA1474 protein
291	AA521149	17767	KIAA1554	KIAA1554 protein
292	N62352	24790	KIAA1573	KIAA1573 protein
293	AW976121	301444	KIAA1673	KIAA1673

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294	AI890497	28501	KIAA1754	KIAA1754 protein
295	T78873	9587	KIAA2002	KIAA2002 protein

Table 5 Commonly up-regulated genes in 20 prostate cancers

PRC Assi gnm ent	Accession No.	Hs.	Symbol	Title
function known				
296	X12433	99364	ABHD2	abhydrolase domain containing 2
297	AF039018	135281	ALP	alpha-actinin-2-associated LIM protein
298	AJ130733	128749	AMACR	alpha-methylacyl-CoA racemase
299	J02611	75736	APOD	apolipoprotein D
300	AF071202	139336	ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
301	AA633487	108708	CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2, beta
302	AF001436	12289	CDC42EP2	CDC42 effector protein (Rho GTPase binding) 2
303	BF981201	408061	FABP5	fatty acid binding protein 5 (psoriasis- associated)
304	D14446	107	FGL1	fibrinogen-like 1
305	S82986	820	HOXC6	homeo box C6
306	AF064493	4980	LDB2	LIM domain binding 2
307	AI767296	123655	NPR3	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)
308	AA858162	124673	NCAG1	NCAG1
309	AI805082	303171	OR51E2	olfactory receptor, family 51, subfamily E, member 2 (prostate-specific G protein- coupled receptor)
310	AF045584	18910	POV1	prostate cancer overexpressed gene 1
311	AI298501	21192	SDK1	sidekick homolog 1 (chicken)
312	U80456	27311	SIM2	single-minded homolog 2 (Drosophila)
313	AD001528	89718	SMS	spermine synthase
314	N21096	99291	STXBP6	syntaxin binding protein 6 (amisyn)
315	L15203	82961	TFF3	trefoil factor 3 (intestinal)
function unknown				
316	D14657	81892	KIAA0101	KIAA0101 gene product
317	AI989530	240845	DKFZP434D	DKFZP434D146 protein

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318	NM_01206	128702	20D7-FC4	hypothetical protein 20D7-FC4
	6			
319	AA206763	7991	C20orf102	chromosome 20 open reading frame 102
320	AI700341	110406		ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens]
321	AI003798	23799		Homo sapiens, clone IMAGE:4791783, mRNA

Table 6 Commonly down-regulated genes in 20 prostate cancers

PRC Assi gnm ent	Accession No.	Hs.	Symbol	Title
function known				
322				
323				
322	AI827230	374481	APCDD1	adenomatosis polyposis coli down-regulated 1
323	BF965257	74120	APM2	adipose specific 2
324	AF245505	72157	DKFZp564I1 922	adlican
325	D00017	217493	ANXA2	annexin A2
326	NM_00115	118796 5	ANXA6	annexin A6
327	AK027126	160786	ASS	argininosuccinate synthetase
328	W91908	6079	GALNAC4S- 6ST	B cell RAG associated protein
329	AB004066	171825	BHLHB2	basic helix-loop-helix domain containing, class B, 2
330	M14745	79241	BCL2	B-cell CLL/lymphoma 2
331	S67310	69771	BF	B-factor, properdin
332	M69225	198689	BPAG1	bullous pemphigoid antigen 1, 230/240kDa
333	X63629	2877	CDH3	cadherin 3, type 1, P-cadherin (placental)
334	AF134640	7235	CACNG3	calcium channel, voltage-dependent, gamma subunit 3
335	M94345	82422	CAPG	capping protein (actin filament), gelsolin-like
336	AF035752	139851	CAV2	caveolin 2
337	K01144	84298	CD74	CD74 antigen
338	AI750036	22116	CDC14B	CDC14 cell division cycle 14 homolog B (S. cerevisiae)
339	NM_00299	80420	CX3CL1	chemokine (C-X3-C motif) ligand 1

340	AF000959	110903	CLDN5	claudin 5 (transmembrane protein deleted in velocardiofacial syndrome)
341	NM_001831	75106	CLU	clusterin (SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2)
342	NM_001733	1279	C1R	complement component 1, r subcomponent
343	K02765	284394	C3	complement component 3
344	D13639	75586	CCND2	cyclin D2
345	BF183952	412999	CSTA	cystatin A (stefin A)
346	M62401	82568	CYP27A1	cytochrome P450, family 27, subfamily A, polypeptide 1
347	J04813	104117	CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5
348	X90579	166079	CYP3A5P2	cytochrome P450, family 3, subfamily A, polypeptide 5 pseudogene 2
349	AW956111	79404	D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence
350	AB012955	129867	KIP2	DNA-dependent protein kinase catalytic subunit-interacting protein 2
351	D83407	156007	DSCR1L1	Down syndrome critical region gene 1-like 1
352	L11329	1183	DUSP2	dual specificity phosphatase 2
353	NM_001421	151139	ELF4	E74-like factor 4 (ets domain transcription factor)
354	AW300770	61265	FAM3D	family with sequence similarity 3, member D
355	D84239	111732	FCGBP	Fc fragment of IgG binding protein
356	AF182316	234680	FER1L3	fer-1-like 3, myoferlin (C. elegans)
357	M87770	278581	FGFR2	fibroblast growth factor receptor 2
358	NM_001456	195464	FLNA	filamin A, alpha (actin binding protein 280)
359	L42176	8302	FHL2	four and a half LIM domains 2
360	NM_000165	74471	GJA1	gap junction protein, alpha 1, 43kDa (connexin 43)
361	AW949747	169946	GATA3	GATA binding protein 3
362	NM_002083	2704	GPX2	glutathione peroxidase 2 (gastrointestinal)
363	NM_002084	386793	GPX3	glutathione peroxidase 3 (plasma)

364	AA290738	301961	GSTM1	glutathione S-transferase M1
365	NM_00208	2699	GPC1	glypican 1
	1			
366	M55543	171862	GBP2	guanylate binding protein 2, interferon-inducible
367	AA666119	92287	GBP3	guanylate binding protein 3
368	NM_00018	250651	HF1	H factor 1 (complement)
	6			
369	AA490691	421136	HOXD11	homeo box D11
370	J02770	36602	IF	I factor (complement)
371	S81914	76095	IER3	immediate early response 3
372	AV646610	34853	ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
373	L08488	32309	INPP1	inositol polyphosphate-1-phosphatase
374	M31159	77326	IGFBP3	insulin-like growth factor binding protein 3
375	M59911	265829	ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
376	X52186	85266	ITGB4	integrin, beta 4
377	AF020201	166154	JAG2	jagged 2
378	X14640	74070	KRT13	keratin 13
379	X07696	80342	KRT15	keratin 15
380	M21389	433845	KRT5	keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)
381	X03212	23881	KRT7	keratin 7
382	AF287272	84728	KLF5	Kruppel-like factor 5 (intestinal)
383	Y00711	234489	LDHB	lactate dehydrogenase B
384	U07643	105938	LTF	lactotransferrin
385	M13452	377973	LMNA	lamin A/C
386	D37766	75517	LAMB3	laminin, beta 3
387	L13210	79339	LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein
388	AF002672	152944	LOH11CR2	loss of heterozygosity, 11, chromosomal region 2, gene A
			A	
389	BE621666	296398	LAPTM4B	lysosomal associated protein transmembrane 4 beta
390	L08895	78995	MEF2C	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)
391	AA779709	7457	MAGE-E1	MAGE-E1 protein

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392	M33906	198253	HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1
393	AW298180	2256	MMP7	matrix metalloproteinase 7 (matrilysin, uterine)
394	AF017418	104105	MEIS2	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse)
395	J02854	9615	MYL9	myosin, light polypeptide 9, regulatory
396	AF203032	198760	NEFH	neurofilament, heavy polypeptide 200kDa
397	M12267	75485	OAT	ornithine aminotransferase (gyrate atrophy)
398	U90878	75807	PDLIM1	PDZ and LIM domain 1 (elfin)
399	M22430	76422	PLA2G2A	phospholipase A2, group IIA (platelets, synovial fluid)
400	D00244	77274	PLAU	plasminogen activator, urokinase
401	AL045876	430637	PTGDS	prostaglandin D2 synthase 21kDa (brain)
402	AF043498	423634	PSCA	prostate stem cell antigen
403	NM_00639	278503	RIG	regulated in glioma
	4			
404	NM_00397	194691	RAI3	retinoic acid induced 3
	9			
405	AA173755	301198	ROBO1	roundabout, axon guidance receptor, homolog 1 (Drosophila)
406	AW965789	66450	SENP1	sentrin/SUMO-specific protease
407	M93056	183583	SERPINB1	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1
408	M13690	151242	SERPING1	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1
409	W73992	132792	SDCCAG43	serologically defined colon cancer antigen 43
410	X51441	332053	SAA1	serum amyloid A1
411	NM_00645	288215	STHM	sialyltransferase
	6			
412	AF215636	5944	SLC11A3	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 3
413	U59299	90911	SLC16A5	solute carrier family 16 (monocarboxylic acid transporters), member 5
414	M55531	33084	SLC2A5	solute carrier family 2 (facilitated glucose/fructose transporter), member 5
415	M81635	160483	STOM	stomatin

416	BF514189	345728	SOCS3	suppressor of cytokine signaling 3
417	AI423028	71622	SMARCD3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3
418	AK001617	24948	SNCAIP	synuclein, alpha interacting protein (synphilin)
419	U21847	82173	TIEG	TGFB inducible early growth response
420	M12670	5831	TIMP1	tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)
421	U54831	75248	TOP2B	topoisomerase (DNA) II beta 180kDa
422	NM_00324	2387	TGM4	transglutaminase 4 (prostate)
	1			
423	W72411	137569	TP73L	tumor protein p73-like
424	D88154	103665	VILL	villin-like
425	X63187	2719	WFDC2	WAP four-disulfide core domain 2
426	AF122922	284122	WIF1	WNT inhibitory factor 1
427	AA916688	85155	ZFP36L1	zinc finger protein 36, C3H type-like 1
428	BF055342	326801	ZNF6	zinc finger protein 6 (CMPX1)
	function unknown			
429	AA706316	32343	ZD52F10	hypothetical gene ZD52F10
430	U57961	181304	13CDNA73	hypothetical protein CG003
431	AA709155	104800	FLJ10134	hypothetical protein FLJ10134
432	AK001021	22505	FLJ10159	hypothetical protein FLJ10159
433	AA180145	351270	LOC152485	hypothetical protein LOC152485
434	AA133590	377830	MGC44669	hypothetical protein MGC44669
435	NM_01476	75137	KIAA0193	KIAA0193 gene product
	6			
436	AI741882	278436	KIAA1474	KIAA1474 protein
437	BF431643	15420	KIAA1500	KIAA1500 protein
438	N62352	24790	KIAA1573	KIAA1573 protein
439	T78873	9587	KIAA2002	KIAA2002 protein
440	AK022877	49476		Homo sapiens cDNA FLJ12815 fis, clone NT2RP2002546.
441	AI566720	380045		Homo sapiens cDNA FLJ34528 fis, clone HLUNG2008066.
442	BE885999	397414		Homo sapiens cDNA: FLJ20860 fis, clone ADKA01632.
443	AK025909	288741		Homo sapiens cDNA: FLJ22256 fis, clone HRC02860.
444	AI269367	101307		Homo sapiens HUT11 protein mRNA,

			partial 3' UTR
445	AL050204	28540	Homo sapiens mRNA; cDNA DKFZp586F1223 (from clone DKFZp586F1223)
446	AV733210	367688	Homo sapiens, clone IMAGE:4794726, mRNA
447	AI027791	132296	ESTs
448	BF111819	21470	ESTs
449	AA632025	444752	ESTs
450	BE868254	380149	ESTs
451	AW510657	156044	ESTs
452	AA620628	186486	ESTs
453	AI769569	112472	ESTs
454	T79422	119237	ESTs
455	AI052358	131741	ESTs
456	N95414	55168	ESTs, Weakly similar to neuronal thread protein [Homo sapiens] [H.sapiens]
457	BG163478	405950	ESTs, Weakly similar to BAI1_HUMAN Brain-specific angiogenesis inhibitor 1 precursor [H.sapiens]

Table 7 Up-regulated genes in 10 PINs

PRC	Accession	Hs.	Symbol	Title
Assi	No			
gnm				
ent				
				function known
458	BE466450	50628	AP4S1	adaptor-related protein complex 4, sigma 1 subunit
459	AW612403	293970	ALDH6A1	aldehyde dehydrogenase 6 family, member A1
460	AJ130733	128749	AMACR	alpha-methylacyl-CoA racemase
461	NM_00164	279518	APLP2	amyloid beta (A4) precursor-like protein 2
462	X59066	405985	ATP5A1	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit, isoform 1
463	AF071202	139336	ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
464	AB019038	44592	HMT-1	beta-1,4 mannosyltransferase
465	AF231023	55173	CELSR3	cadherin, EGF LAG seven-pass G-type

466	AI817172	29423	COLEC12	receptor 3 (flamingo homolog, <i>Drosophila</i>)
467	Z21488	143434	CNTN1	collectin sub-family member 12
468	AF255443	268281	CRNKL1	contactin 1
469	NM_00543	288862	D10S170	Crn, crooked neck-like 1 (<i>Drosophila</i>)
	6			DNA segment on chromosome 10 (unique) 170
470	AI697792	21189	DNAJA2	DnaJ (Hsp40) homolog, subfamily A, member 2
471	AF039918	80975	ENTPD5	ectonucleoside triphosphate diphosphohydrolase 5
472	AF176699	49526	FBXL4	F-box and leucine-rich repeat protein 4
473	M99487	1915	FOLH1	folate hydrolase (prostate-specific membrane antigen) 1
474	NM_00015	184141	GCDH	glutaryl-Coenzyme A dehydrogenase
	9			
475	AW967035	159572	HS3ST3B1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1
476	NM_00533	211571	HCCS	holocytochrome c synthase (cytochrome c heme-lyase)
477	U26726	1376	HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2
478	U89281	11958	RODH	3-hydroxysteroid epimerase
479	U42408	18141	LAD1	ladinin 1
480	L25931	152931	LBLR	lamin B receptor
481	Z30137	49998	LDB3	LIM domain binding 3
482	AF001174	57732	MAPK11	mitogen-activated protein kinase 11
483	M92449	264330	ASAHL	N-acylsphingosine amidohydrolase (acid ceramidase)-like
484	W23499	118654	ASAHL	N-acylsphingosine amidohydrolase (non-lysosomal ceramidase) 2
485	R22536	220324	FLJ13052	NAD kinase
486	AA704060	8248	NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)
487	AI805082	303171	OR51E2	olfactory receptor, family 51, subfamily E, member 2
488	AK025460	286049	PSA	phosphoserine aminotransferase
489	NM_02120	380812	PLEKHB1	pleckstrin homology domain containing, family B (ejectins) member 1
490	AI346354	75871	PRKCBP1	protein kinase C binding protein 1

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491	AF044588	344037	PRC1	protein regulator of cytokinesis 1
492	NM_01234	78776	NMA	putative transmembrane protein
2				
493	AL041152	13264	RC3	rabconnectin-3
494	L10333	99947	RTN1	reticulon 1
495	M32313	552	SRD5A1	steroid-5-alpha-reductase, alpha polypeptide 1
496	U04735	352341	STCH	stress 70 protein chaperone, microsome-associated, 60kDa
497	U66035	125565	TIMM8A	translocase of inner mitochondrial membrane 8 homolog A (yeast)
498	AA907673	432605	UGCG	UDP-glucose ceramide glucosyltransferase
499	AA164237	279840	ZNF222	zinc finger protein 222
500	NM_00630	193583	ZNF230	zinc finger protein 230
0				
	function unknown			
501	AK023414	22972	FLJ13352	hypothetical protein FLJ13352
502	AI341472	274337	FLJ20666	hypothetical protein FLJ20666
503	N48613	311163	FLJ30162	hypothetical protein FLJ30162
504	BG179141	7962	FLJ30525	hypothetical protein FLJ30525
505	AW971484	105069	LOC148418	hypothetical protein LOC148418
506	AK000569	107444	LOC90075	hypothetical protein LOC90075
507	D43948	76989	KIAA0097	KIAA0097 gene product
508	AB011085	301658	KIAA0513	KIAA0513 gene product
509	AB011127	43107	KIAA0555	KIAA0555 gene product
510	AI151160	155983	KIAA0677	KIAA0677 gene product
511	T55178	9846	KIAA1040	KIAA1040 protein
512	AI094513	21896	KIAA1136	KIAA1136 protein
513	AA206763	7991	C20orf102	chromosome 20 open reading frame 102
514	AF131828	7961	C9orf25	chromosome 9 open reading frame 25
515	AA825819	7535	LOC55871	LOC55871
516	AW135763	6375	HT010	uncharacterized hypothalamus protein HT010
517	AK025329	7158		DKFZP566H073 protein
518	AL390127	433788		Homo sapiens mRNA; cDNA DKFZp761P06121
519	AI074176	31535		Homo sapiens, clone IMAGE:3460742, mRNA, partial cds
520	AI133467	95612		ESTs
521	BF514823	119065		ESTs

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522	AA897408	190065	ESTs
523	AI478401	104591	ESTs
524	AA430571	104881	ESTs
525	AA521342	101428	ESTs
526	N62332	102728	ESTs
527	H17800	438858	ESTs
528	AA826048	117887	ESTs
529	AA677094	117035	ESTs
530	AA682521	117261	ESTs
531	AI554006	112694	ESTs
532	AI004966	445098	ESTs
533	N52767	23406	EST
534	BF109251	353121	ESTs, Weakly similar to hypothetical protein FLJ20378
535	AI700341	110406	ESTs, Weakly similar to hypothetical protein FLJ20489
536	AA743154	373991	ESTs, Weakly similar to neuronal thread protein
537	AI352507	263600	ESTs, Weakly similar to RL17_HUMAN 60S ribosomal protein L17 (L23)

Table 8 Down-regulated Genes in 10 PINs

PRC	Accession	Hs.	Symbol	Title
Assi	No			
gnm				
ent				
				function known
538	K03000	76392	ALDH1A1	aldehyde dehydrogenase 1 family, member A1
539	AF055024	153489	ASB1	ankyrin repeat and SOCS box-containing 1
540	M81844	87268	ANXA8	annexin A8
541	X82206	153961	ACTR1A	ARP1 actin-related protein 1 homolog A, centractin alpha (yeast)
542	AW014316	1578	BIRC5	baculoviral IAP repeat-containing 5 (survivin)
543	S67310	69771	BF	B-factor, properdin
544	AF132972	279772	CGI-38	brain specific protein
545	BE826171	100686	BCMP11	breast cancer membrane protein 11
546	AF134640	7235	CACNG3	calcium channel, voltage-dependent, gamma subunit 3

547	AF177775	76688	CES1	carboxylesterase 1 (monocyte/macrophage serine esterase 1)
548	Z18951	74034	CAV1	caveolin 1, caveolae protein, 22kDa
549	K01144	84298	CD74	CD74 antigen
550	NM_00299	80420	CX3CL1	chemokine (C-X3-C motif) ligand 1
	6			
551	U58514	154138	CHI3L2	chitinase 3-like 2
552	W19536	363572	CEPT1	choline/ethanolaminephosphotransferase
553	U16306	81800	CSPG2	chondroitin sulfate proteoglycan 2 (versican)
554	AF101051	7327	CLDN1	claudin 1
555	AI150272	258811	COPG2	coatomer protein complex, subunit gamma 2
556	AV712344	285401	CSF2RB	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
557	NM_00173	1279	C1R	complement component 1, r subcomponent
	3			
558	K02765	284394	C3	complement component 3
559	AF081287	4076	CTDP1	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) phosphatase, subunit 1
560	L12579	147049	CUTL1	cut-like 1, CCAAT displacement protein (<i>Drosophila</i>)
561	D86977	78054	DDX38	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 38
562	M26602	274463	DEFA1	defensin, alpha 1, myeloid-related sequence
563	AF097021	273321	GW112	differentially expressed in hematopoietic lineages
564	NM_00618	71891	DDR2	discoidin domain receptor family, member 2
	2			
565	NM_00195	73946	ECGF1	endothelial cell growth factor 1 (platelet- derived)
	3			
566	BF981201	408061	FABP5	fatty acid binding protein 5 (psoriasis- associated)
567	AF112152	11494	FBLN5	fibulin 5
568	U60115	239069	FHL1	four and a half LIM domains 1
569	NM_00208	2704	GPX2	glutathione peroxidase 2 (gastrointestinal)
	3			
570	NM_00208	386793	GPX3	glutathione peroxidase 3 (plasma)
	4			

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571	NM_00208	2699	GPC1	glypican 1
	1			
572	AI887814	4953	GOLGA3	golgi autoantigen, golgin subfamily a, 3
573	D21239	9195	GRF2	guanine nucleotide-releasing factor 2 (specific for crk proto-oncogene)
574	NM_00630	41707	HSPB3	heat shock 27kDa protein 3
	8			
575	AK001601	69594	HMG20A	high-mobility group 20A
576	J02770	36602	IF	I factor (complement)
577	S81914	76095	IER3	immediate early response 3
578	AI922295	413826	IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)
579	X67301	153261	IGHM	immunoglobulin heavy constant mu
580	AW518944	76325	IGJ	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides
581	AK026991	61790	IPO4	importin 4
582	M31159	77326	IGFBP3	insulin-like growth factor binding protein 3
583	AK026736	57664	ITGB6	integrin, beta 6
584	NM_00219	80645	IRF1	interferon regulatory factor 1
	8			
585	U72882	50842	IFI35	interferon-induced protein 35
586	M13143	1901	KLKB1	kallikrein B, plasma (Fletcher factor) 1
587	U07643	105938	LTF	lactotransferrin
588	AF025534	77062	LILRB5	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 5
589	AI563896	1569	LHX2	LIM homeobox protein 2
590	AA644276	102267	LOX	lysyl oxidase
591	M81141	73931	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1
592	BF697545	365706	MGP	matrix Gla protein
593	NM_00453	111301	MMP2	matrix metalloproteinase 2 (gelatinase A)
	0			
594	AW298180	2256	MMP7	matrix metalloproteinase 7 (matrilysin)
595	NM_00592	3745	MFGE8	milk fat globule-EGF factor 8 protein
	8			
596	AI023878	406591	MTIF3	mitochondrial translational initiation factor 3
597	J05581	89603	MUC1	mucin 1, transmembrane

598	M94132	315	MUC2	mucin 2, intestinal/tracheal
599	AJ293659	12909	MCOLN1	mucolipin 1
600	J02854	9615	MYL9	myosin, light polypeptide 9, regulatory
601	AB037787	26229	NLGN2	neuroligin 2
602	NM_00616	364345	NNMT	nicotinamide N-methyltransferase
	9			
603	S51033	79396	MPG	N-methylpurine-DNA glycosylase
604	N35034	8121	NOTCH2	Notch homolog 2 (Drosophila)
605	NM_00616	75643	NFE2	nuclear factor (erythroid-derived 2), 45kDa
606	AW949776	3187	NFX1	nuclear transcription factor, X-box binding 1
607	M13692	572	ORM1	orosomucoid 1
608	BF115519	14125	PA26	p53 regulated PA26 nuclear protein
609	L03203	103724	PMP22	peripheral myelin protein 22
610	AA398096	198278	PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4
611	AI660921	107125	PLVAP	plasmalemma vesicle associated protein
612	D29833	2207	PROL3	proline rich 3
613	N26005	303090	PPP1R3C	protein phosphatase 1, regulatory (inhibitor) subunit 3C
614	BF673741	71119	N33	Putative prostate cancer tumor suppressor
615	AI004873	198281	PKM2	pyruvate kinase, muscle
616	H46145	27744	RAB3A	RAB3A, member RAS oncogene family
617	AK026092	180040	RIN3	Ras and Rab interactor 3
618	BG054844	6838	ARHE	ras homolog gene family, member E
619	NM_00397	194691	RAI3	retinoic acid induced 3
	9			
620	AA927661	201675	RBMS5	RNA binding motif protein 5
621	BF027943	2962	S100P	S100 calcium binding protein P
622	AI719545	278431	SCO2	SCO cytochrome oxidase deficient homolog 2 (yeast)
623	X16150	82848	SELL	selectin L (lymphocyte adhesion molecule 1)
624	J05176	234726	SERPINA3	serine (or cysteine) proteinase inhibitor, clade A, member 3
625	BF126636	332053	SAA1	serum amyloid A1
626	NM_00417	1575	SNRPD3	small nuclear ribonucleoprotein D3
	5			polypeptide 18kDa
627	AF036109	193665	SLC28A2	solute carrier family 28 (sodium-coupled nucleoside transporter), member 2

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628	AF058918	5699	SEDLP	spondyloepiphyseal dysplasia, late, pseudogene
629	NM_000348	1989	SRD5A2	steroid-5-alpha-reductase, alpha polypeptide 2
630	AF059203	20580	SOAT2	sterol O-acyltransferase 2
631	AA853967	124574	TAS1R1	taste receptor, type 1, member 1
632	AF082185	8375	TRAF4	TNF receptor-associated factor 4
633	AI091425	9030	TONDU	TONDU
634	AA682533	44269	TRIPIN	tripin
635	AB025254	283761	PCTAIRE2B	tudor repeat associator with PCTAIRE 2 P
636	D17517	301	TYRO3	TYRO3 protein tyrosine kinase
637	AF000993	13980	UTX	ubiquitously transcribed tetratricopeptide repeat gene, X chromosome
638	AW574558	121102	VNN2	vanin 2
639	H20162	2126	VIPR2	vasoactive intestinal peptide receptor 2
640	BE382636	25960	MYCN	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
641	X63187	2719	WFDC2	WAP four-disulfide core domain 2
	function unknown			
642	AI042017	23756	C1orf13	chromosome 1 open reading frame 13
643	AA614050	267566	C14orf58	chromosome 14 open reading frame 58
644	AK023453	334721	FLJ13391	hypothetical protein FLJ13391
645	BE465676	353196	FLJ14564	hypothetical protein FLJ14564
646	AK026924	105642	FLJ21936	hypothetical protein FLJ21936
647	AW195243	108812	FLJ22004	hypothetical protein FLJ22004
648	BF965831	135121	FLJ22415	hypothetical protein FLJ22415
649	AK026486	118183	FLJ22833	hypothetical protein FLJ22833
650	AW271223	5890	FLJ23306	hypothetical protein FLJ23306
651	AI359551	22015	FLJ90119	hypothetical protein FLJ90119
652	BG054529	206501	LOC57228	hypothetical protein from clone 643
653	AI149729	120557	LOC285286	hypothetical protein LOC285286
654	AI089621	22051	MGC15548	hypothetical protein MGC15548
655	AW005320	236547	MGC22916	hypothetical protein MGC22916
656	AI076840	40808	MGC33926	hypothetical protein MGC33926
657	AW340131	56382	FLJ32384	hypothetical protein MGC39389
658	AK025996	209614	MGC4415	hypothetical protein MGC4415
659	AA827188	351605	MGC45417	hypothetical protein MGC45417
660	H04833	6336	KIAA0672	KIAA0672 product
661	AB033103	6385	KIAA1277	KIAA1277 protein
662	BG054798	26204	KIAA1295	KIAA1295 protein

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663	AI694131	29002	KIAA1706	KIAA1706 protein
664	AL137345	298850	KIAA1936	KIAA1936 protein
665	AK025585	380169		DKFZp727A071
666	AB037861	112184		DKFZP586J0619 protein
667	W58516	12396		Homo sapiens cDNA FLJ33095 fis, clone TRACH2000708.
668	AW967916	31944		Homo sapiens cDNA FLJ33236 fis, clone ASTRO2002571.
669	AF052090	106620		Homo sapiens clone 23950 mRNA sequence
670	AL110236	321022		Homo sapiens mRNA; cDNA DKFZp566P1124 (from clone DKFZp566P1124)
671	BE348293	29283		Homo sapiens proteoglycan link protein mRNA, complete cds.
672	AI139601	120590		Homo sapiens, clone IMAGE:5750475, mRNA
673	H42381	348805		hypothetical protein DKFZp667B0210
674	AA180005	115029		ESTs
675	AA648546	230703		ESTs
676	AI916303	7444		ESTs
677	AA700898	113117		ESTs
678	AI246644	259679		ESTs
679	AI807279	443735		ESTs
680	AI160304	28313		ESTs
681	AA768888	446195		ESTs
682	BE502928	445376		ESTs
683	AA568515	293510		ESTs
684	AI732560	215976		ESTs
685	AI821961	126215		ESTs
686	AA928743	132527		ESTs
687	AA910771	130421		ESTs
688	AA938326	127167		ESTs
689	AA897581	445725		ESTs
690	AA004313	446619		ESTs, Highly similar to HIRA-interacting protein 3
691	H21968	285520		ESTs, Moderately similar to hypothetical protein FLJ20489
692	AI223250	131365		ESTs, Weakly similar to T31613 hypothetical protein Y50E8A.i - Caenorhabditis elegans]

Table 2 Primer sequences for semi-quantitative RT-PCR experiments

Symbol	Forward primer	SEQ.ID .NO.	Reverse primer	SEQ.ID .NO.
AMACR	5'-TCATGATCTCCC TCTAACGACAT-3'	1	5'-TGTTGCTGTGTGTTG GGTATAAG-3'	2
HOXC6	5'-CCTGGGGGTCA TTATGGCATTTC-3'	3	5'-TTCTCCTACTGGCTA AACAAACG-3'	4
POV1	5'-GGTGCCTCTTAT CTCCTTCT-3'	5	5'-CTTCCTTTTATTTC CTCT-3'	6
ABHD2	5'-GTACTGGCTTA AAAGCAACCAG-3'	7	5'-CTCAGTGACCTGGAT CTGACCT-3'	8
C20ORF 102	5'-AACCACTTCTTG CGAGTCCTT-3'	9	5'-TATTCAGGTTGGCTG GTAGTCAC-3'	10
β-actin	5'-TTGGCTTGACTC AGGATTAA-3'	11	5'-TGGACTTGGGAGAGG ACTGG-3'	12

Industrial Applicability

The gene-expression analysis of PRC and PIN described herein, obtained through a combination of laser-capture dissection and genome-wide cDNA microarray, has identified 5 specific genes as targets for cancer prevention and therapy. Based on the expression of a subset of these differentially expressed genes, the present invention provides a molecular diagnostic markers for identifying or detecting either or both of PRC and PIN.

The methods described herein are also useful in the identification of additional molecular targets for prevention, diagnosis and treatment of either or both of PRC and PIN. 10 The data reported herein add to a comprehensive understanding of PRC, facilitate development of novel diagnostic strategies, and provide clues for identification of molecular targets for therapeutic drugs and preventative agents. Such information contributes to a more profound understanding of prostatic tumorigenesis, and provide indicators for developing novel strategies for diagnosis, treatment, and ultimately 15 prevention of PRC.

All patents, patent applications, and publications cited herein are incorporated by reference in their entirety. Furthermore, while the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the 20 spirit and scope of the invention.

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CLAIMS

1. A method of diagnosing either or both of PRC and PIN or a predisposition to developing either or both of PRC and PIN in a subject, comprising determining a level of expression of a PRC -associated gene in a patient derived biological sample, wherein an increase or decrease of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing either or both of PRC and PIN.
2. The method of claim 1, wherein said PRC -associated gene is selected from the group consisting of PRC 1-88, wherein an increase in said level compared to a normal control level indicates said subject suffers from or is at risk of developing either or both of PRC and PIN.
3. The method of claim 2, wherein said increase is at least 10% greater than said normal control level.
4. The method of claim 1, wherein said PRC -associated gene is selected from the group consisting of PRC 89-295, wherein a decrease in said level compared to a normal control level indicates said subject suffers from or is at risk of developing either or both of PRC and PIN.
5. The method of claim 4, wherein said decrease is at least 10% lower than said normal control level.
6. The method of claim 1, wherein said method further comprises determining said level of expression of a plurality of PRC -associated genes.
7. The method of claim 1, wherein the expression level is determined by any one method select from group consisting of:
 - (a) detecting the mRNA of the PRC -associated genes,
 - (b) detecting the protein encoded by the PRC -associated genes, and
 - (c) detecting the biological activity of the protein encoded by the PRC -associated genes,
8. The method of claim 1, wherein said level of expression is determined by detecting hybridization of a PRC -associated gene probe to a gene transcript of said patient-

derived biological sample.

9. The method of claim 8, wherein said hybridization step is carried out on a DNA array.
10. The method of claim 1, wherein said biological sample comprises an epithelial cell.
- 5 11. The method of claim 1, wherein said biological sample comprises either or both of PRC and PIN cell.
12. The method of claim 8, wherein said biological sample comprises an epithelial cell from a PRC or PIN.
13. A PRC reference expression profile, comprising a pattern of gene expression of two 10 or more genes selected from the group consisting of PRC 1-295.
14. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 1-88.
15. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 89-295.
- 15 16. A method of screening for a compound for treating or preventing either or both of PRC and PIN, said method comprising the steps of:
 - a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-295;
 - b) detecting the binding activity between the polypeptide and the test compound;
 - 20 and
 - c) selecting a compound that binds to the polypeptide.
17. A method of screening for a compound for treating or preventing either or both of PRC and PIN, said method comprising the steps of:
 - a) contacting a candidate compound with a cell expressing one or more marker genes, 25 wherein the one or more marker genes is selected from the group consisting of PRC 1-295; and
 - b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 1-88, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 89-295.

18. A method of screening for a compound for treating or preventing either or both of PRC and PIN, said method comprising the steps of:
 - a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-295;
 - 5 b) detecting the biological activity of the polypeptide of step (a); and
 - c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88 in comparison with the biological activity detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 89-295 in comparison 10 with the biological activity detected in the absence of the test compound.
19. The method of claim 17, wherein said cell comprises a either or both of PRC and PIN cell.
20. A method of screening for compound for treating or preventing either or both of PRC and PIN, said method comprising the steps of:
 - a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PRC 1-295
 - 20 b) measuring the activity of said reporter gene; and
 - c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PRC 1-88 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 89-295, as compared to a control.
21. A kit comprising a detection reagent which binds to two or more nucleic acid sequences selected from the group consisting of PRC 1-295.
22. An array comprising a nucleic acid which binds to two or more nucleic acid sequences selected from the group consisting of PRC 1-295.
- 30 23. A method of treating or preventing either or both of PRC and PIN in a subject

comprising the step of administering to said subject a compound that decreases the expression or activity of a polypeptide encoded by a gene selected from the group consisting of PRC 1-88.

24. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject an antisense nucleic composition, said composition comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of PRC 1-88.
- 5 25. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence selected from the group consisting of PRC 1-88.
- 10 26. A method for treating or preventing either or both of PRC and PIN in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 1-88.
- 15 27. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.
- 20 28. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a compound that increases the expression or activity of PRC 89-295
29. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 89-295, or polypeptide encoded thereby.
- 25 30. A method for treating or preventing either or both of PRC and PIN in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 16-20.

31. A composition for treating or preventing either or both of PRC and PIN, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide select from group consisting of PRC 1-88 as an active ingredient, and a pharmaceutically acceptable carrier.
5
32. A composition for treating or preventing either or both of PRC and PIN, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 1-88 as an active ingredient, and a pharmaceutically acceptable carrier.
10
33. A composition for treating or preventing either or both of PRC and PIN, said composition comprising a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 89-295, or polypeptide encoded thereby as an active ingredient, and a pharmaceutically acceptable carrier.
15
34. A composition for treating or preventing either or both of PRC and PIN, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 16-20 as an active ingredient, and a pharmaceutically acceptable carrier.
20
35. A method of diagnosing PRC or a predisposition to developing PRC in a subject, comprising determining a level of expression of a PRC -associated gene in a patient derived biological sample, wherein an increase or decrease of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing PRC.
25
36. The method of claim 35, wherein said PRC -associated gene is selected from the group consisting of PRC 296-321, wherein an increase in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PRC.
30
37. The method of claim 36, wherein said increase is at least 10% greater than said normal control level.
38. The method of claim 35, wherein said PRC -associated gene is selected from the

group consisting of PRC 322-457, wherein a decrease in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PRC.

39. The method of claim 38, wherein said decrease is at least 10% lower than said normal control level.
- 5 40. The method of claim 35, wherein said method further comprises determining said level of expression of a plurality of PRC -associated genes.
41. The method of claim 35, wherein the expression level is determined by any one method select from group consisting of:
 - 10 (a) detecting the mRNA of the PRC -associated genes,
 - (b) detecting the protein encoded by the PRC -associated genes, and
 - (c) detecting the biological activity of the protein encoded by the PRC -associated genes,
42. The method of claim 35, wherein said level of expression is determined by detecting hybridization of a PRC -associated gene probe to a gene transcript of said patient-derived biological sample.
- 15 43. The method of claim 42, wherein said hybridization step is carried out on a DNA array.
44. The method of claim 35, wherein said biological sample comprises an epithelial cell.
- 20 45. The method of claim 35, wherein said biological sample comprises PRC cell.
46. The method of claim 42, wherein said biological sample comprises an epithelial cell from a PRC.
47. A PRC reference expression profile, comprising a pattern of gene expression of two 25 or more genes selected from the group consisting of PRC 296-457.
48. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 296-321.
49. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 322-457.

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50. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:
 - a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 296-457;
 - 5 b) detecting the binding activity between the polypeptide and the test compound; and
 - c) selecting a compound that binds to the polypeptide.
51. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:
 - 10 a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 296-457; and
 - b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 296-321, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 322-457.
52. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:
 - 20 a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 296-457;
 - b) detecting the biological activity of the polypeptide of step (a); and
 - c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 296-321 in comparison with the biological activity detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 322-457 in comparison with the biological activity detected in the absence of the test compound.
- 25
53. The method of claim 51, wherein said cell comprises a PRC cell.
54. A method of screening for compound for treating or preventing PRC, said method comprising the steps of:
 - 30

- 5 a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PRC 296-457
- 10 b) measuring the activity of said reporter gene; and
- 15 c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PRC 296-321 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 322-457, as compared to a control.
- 20 55. A kit comprising a detection reagent which binds to two or more nucleic acid sequences selected from the group consisting of PRC 296-457.
- 25 56. An array comprising a nucleic acid which binds to two or more nucleic acid sequences selected from the group consisting of PRC 296-457.
- 30 57. A method of treating or preventing PRC in a subject comprising the step of administering to said subject a compound that decreases the expression or activity of a polypeptide encoded by a gene selected from the group consisting of PRC 296-321
- 35 58. A method of treating or preventing PRC in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of PRC 296-321.
- 40 59. A method of treating or preventing PRC in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence selected from the group consisting of PRC 296-321.
- 45 60. A method for treating or preventing PRC in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 296-321.

61. A method of treating or preventing PRC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 296-321 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.
- 5 62. A method of treating or preventing PRC in a subject comprising administering to said subject a compound that increases the expression or activity of PRC 322-457
63. A method of treating or preventing PRC in a subject comprising administering to said subject a pharmaceutically effective amount of polynucleotide selected from the group consisting of PRC 322-457, or polypeptide encoded thereby.
- 10 64. A method for treating or preventing PRC in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 50-54.
65. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide selected from the group consisting of PRC 296-321 as an active ingredient, and a pharmaceutically acceptable carrier.
- 15 66. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 296-321 as an active ingredient, and a pharmaceutically acceptable carrier.
- 20 67. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of polynucleotide selected from the group consisting of PRC 322-457, or polypeptide encoded thereby as an active ingredient, and a pharmaceutically acceptable carrier.
- 25 68. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 50-54 as an active ingredient, and a pharmaceutically acceptable carrier.
69. A method of diagnosing PIN or a predisposition to developing PIN in a subject, comprising determining a level of expression of a PRC -associated gene in a patient

derived biological sample, wherein an increase or decrease of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing PIN.

70. The method of claim 69, wherein said PRC -associated gene is selected from the group consisting of PRC 458-537, wherein an increase in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PIN.
- 5 71. The method of claim 70, wherein said increase is at least 10% greater than said normal control level.
- 10 72. The method of claim 69, wherein said PRC -associated gene is selected from the group consisting of PRC 538-692, wherein a decrease in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PIN.
- 15 73. The method of claim 72, wherein said decrease is at least 10% lower than said normal control level.
74. The method of claim 69, wherein said method further comprises determining said level of expression of a plurality of PRC -associated genes.
75. The method of claim 69, wherein the expression level is determined by any one method select from group consisting of:
 - 20 (a) detecting the mRNA of the PRC -associated genes,
 - (b) detecting the protein encoded by the PRC -associated genes, and
 - (c) detecting the biological activity of the protein encoded by the PRC -associated genes,
76. The method of claim 69, wherein said level of expression is determined by
 - 25 detecting hybridization of a PRC -associated gene probe to a gene transcript of said patient-derived biological sample.
77. The method of claim 76, wherein said hybridization step is carried out on a DNA array.
78. The method of claim 69, wherein said biological sample comprises an epithelial cell.

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79. The method of claim 69, wherein said biological sample comprises PIN cell.
80. The method of claim 76, wherein said biological sample comprises an epithelial cell from a PIN.
81. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 458-692.
82. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 458-537.
83. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 538-692.
84. A method of screening for a compound for treating or preventing PIN or preventing PRC, said method comprising the steps of:
 - a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 458-692;
 - b) detecting the binding activity between the polypeptide and the test compound;
 - and
 - c) selecting a compound that binds to the polypeptide.
85. A method of screening for a compound for treating or preventing PIN or preventing PRC, said method comprising the steps of:
 - a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 458-692; and
 - b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 458-537, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 538-692.
86. A method of screening for a compound for treating or preventing PIN or preventing PRC, said method comprising the steps of:
 - a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 458-692;

5 b) detecting the biological activity of the polypeptide of step (a); and
c) selecting a compound that suppresses the biological activity of the polypeptide
encoded by a nucleic acid selected from the group consisting of PRC 458-537 in
comparison with the biological activity detected in the absence of the test
compound, or enhances the the biological activity of the polypeptide encoded by
a nucleic acid selected from the group consisting of PRC 538-692 in comparison
with the biological activity detected in the absence of the test compound.

10 87. The method of claim 85, wherein said cell comprises a PIN cell.

15 88. A method of screening for compound for treating or preventing PIN, or preventing
PRC, said method comprising the steps of:
a) contacting a candidate compound with a cell into which a vector comprising the
transcriptional regulatory region of one or more marker genes and a reporter gene
that is expressed under the control of the transcriptional regulatory region has
been introduced, wherein the one or more marker genes are selected from the
group consisting of PRC 458-692

20 b) measuring the activity of said reporter gene; and
c) selecting a compound that reduces the expression level of said reporter gene when
said marker gene is an up-regulated marker gene selected from the group
consisting of PRC 458-537 or that enhances the expression level of said reporter
gene when said marker gene is a down-regulated marker gene selected from the
group consisting of PRC 538-692, as compared to a control.

25 89. A kit comprising a detection reagent which binds to two or more nucleic acid
sequences selected from the group consisting of PRC 458-692.

90. An array comprising a nucleic acid which binds to two or more nucleic acid
25 sequences selected from the group consisting of PRC 458-692.

91. A method of treating or preventing PIN or preventing PRC in a subject comprising
the step of administering to said subject a compound that decreases the expression
or activity of a polypeptide encoded by a gene selected from the group consisting of
PRC 458-537.

30 92. A method of treating or preventing PIN or preventing PRC in a subject comprising
administering to said subject an antisense composition, said composition

comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of PRC 458-537.

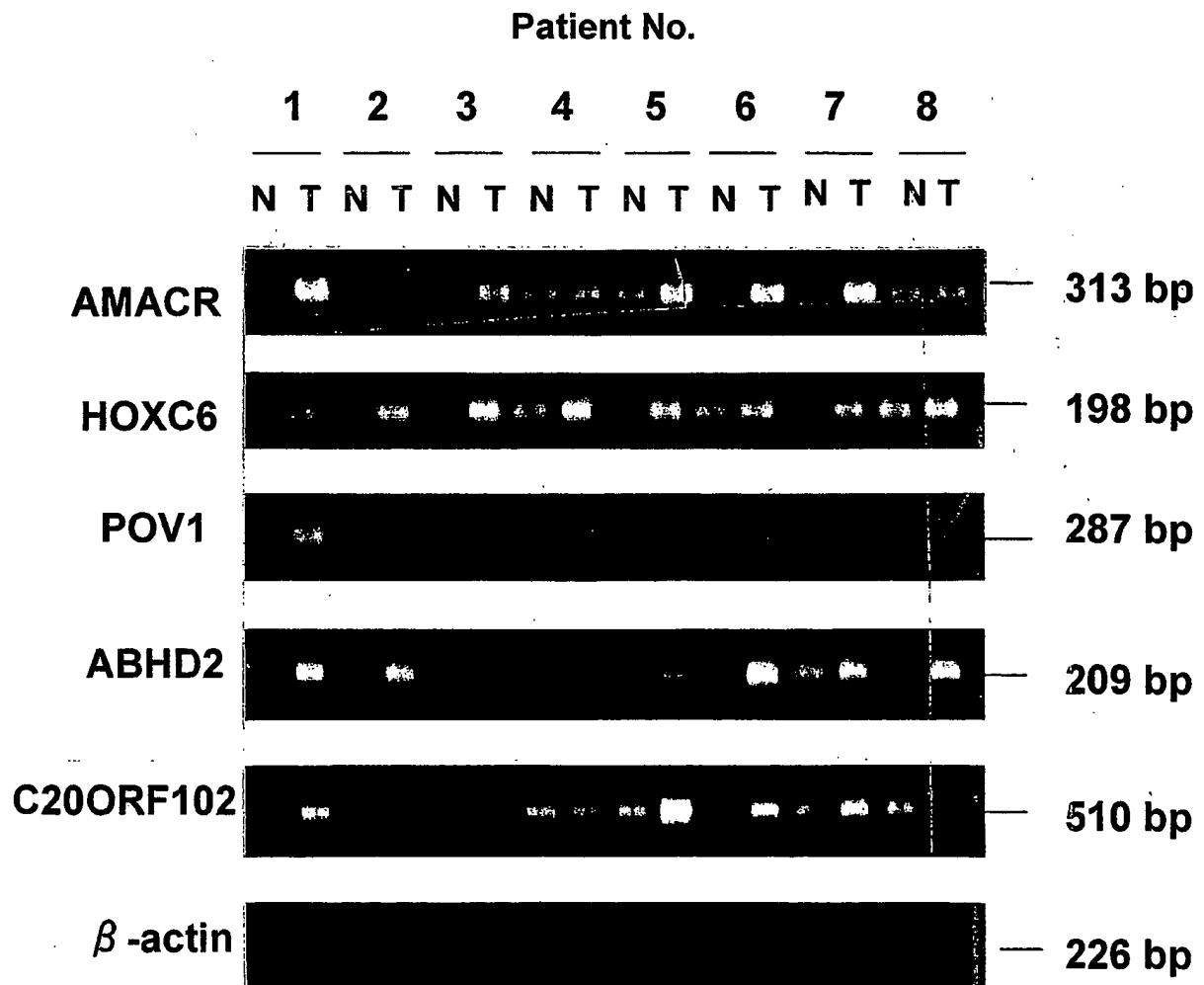
93. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence selected from the group consisting of PRC 458-537.
94. A method for treating or preventing PIN or preventing PRC in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 458-537.
95. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 458-537 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.
96. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject a compound that increases the expression or activity of PRC 538-692
97. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 538-692, or polypeptide encoded thereby.
98. A method for treating or preventing PIN or preventing PRC in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 84-88.
99. A composition for treating or preventing PIN or preventing PRC, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide select from group consisting of PRC 458-537 as an active ingredient, and a pharmaceutically acceptable carrier.
100. A composition for treating or preventing PIN or preventing PRC, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof

that binds to a protein encoded by any one gene selected from the group consisting of PRC 458-537 as an active ingredient, and a pharmaceutically acceptable carrier.

101. A composition for treating or preventing PIN or preventing PRC, said composition comprising a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 538-692, or polypeptide encoded thereby as an active ingredient, and a pharmaceutically acceptable carrier.
102. A composition for treating or preventing PIN or preventing PRC, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 84-88 as an active ingredient, and a pharmaceutically acceptable carrier.

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FIG.1



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SEQUENCE LISTING

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JAPAN AS REPRESENTED BY THE PRESIDENT OF THE UNIVERSITY OF TOKYO

<120> METHOD FOR DIAGNOSING PROSTATE CANCER

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<151> 2002-09-30

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7 / 7

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